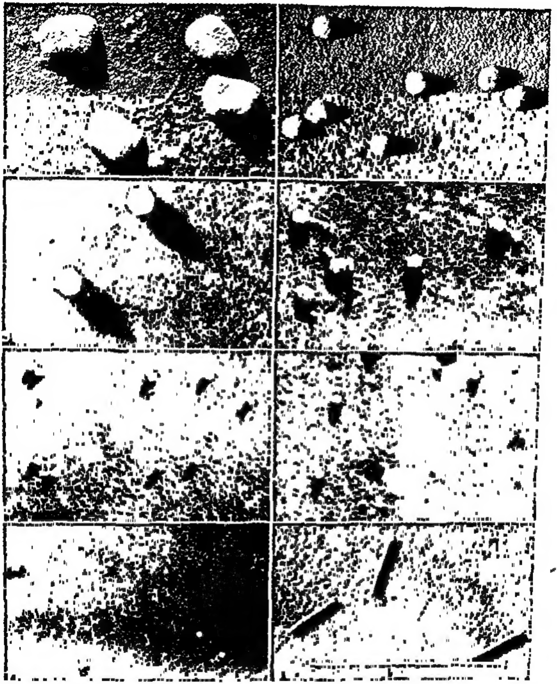
VIRAL AND RICKETTSIAL INFECTIONS OF MAN



Electronmicrographs of 8 viruses shown at the same magnification (×50,000). (1) vaccinia virus, (2) PR8 influenza virus, (3) cytoplasmic virus of *Tipula paludosa* (R C Williams and K. M. Smith, unpublished), (4) T4 bacteriophage, (5) T3 bacteriophage, (6) Shope papilloma virus; (7) poliomyelitis virus; (8) tobacco mosaic virus Micrographs 3 to 7 show frozen-dried preparations [Micrographs by R. C Williams, unpublished, virus preparations, (1) by R C Williams, (2), (6) and (8) by C. A Knight, (4) and (5) by D Fraser; (7) by C E Schwerdt and F. L. Schaffer, all of the Virus Laboratory, University of California, Berkeley]

VIRAL AND RICKETTSIAL INFECTIONS OF MAN

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Preface

So much has been learned of viral and rickettsial infections during the 6 years since the second edition of this book was published as to make the need for a new edition almost imperative In this short interval, several new groups of viruses have been discovered, and the recognition of their position in relation to a variety of infectious processes has gradually unfolded Several viruses, which were known earlier, have been found to have common properties that were previously unsuspected and to possess common antigenic components which have necessitated their reclassification in definite groups Tissue cultures have emerged as highly valuable and widely useful materials for the investigation of viral agents, and the discovery of the cytopathogenic effects of many viruses has resulted in the development of technics which in several instances make the use of intact animals less rewarding than the employment of in vitro procedures Important changes in several conceptual schemes have appeared because of the acquisition and the integration of new information on a broad base Some unifying concepts have been developed, particularly regarding the mechanism of virus multiplication, which serve to diminish if not to abolish the significance of differences between various categories based largely on distinctions among their host species

All of the contributors to this book have concurred with the view of the editors that, because of the remarkable growth of knowledge bearing on viral and rickettsial infections, an attempt at revision of the second edition would undoubtedly have been inadequate Therefore, they have graciously undertaken the tedious and time-consuming task of preparing an entirely new book

This edition is the result of the efforts of 44 contributors, some 14 more than participated in the preparation of the second edition All of the contributors were selected because of their eminence and experience in this field of investigation and because of their special fitness to describe and discuss authoritatively the knowledge available in a particular sector The editors have not attempted to delineate the material considered or the information covered in the several chapters and have carefully avoided influencing either the views or the conclusions of the authors In spite of the full freedom of the contributors to write as they thought best, it is remarkable how small and infrequent are the areas of disagreement and how minor the issues when occasionally they occur

The present volume contains 46 chapters some 7 more than the second edition Three of the new chapters deal with common features of viruses and the infections they induce These are· "Biochemistry of the Virus-Infected Cell," "Virus-Host Cell Relation" and "Chemotherapy and Virus Infection" Two other new chapters are devoted to groups of viruses that have been discovered since the second edition appeared ECHO viruses and Adenoviruses The earlier chapter on "Viral Encephalitides" has been supplanted by 4 chapters concerning "Arthropod-Borne Animal Viruses" and the infections they induce The previous chapter on poliomyelitis has been expanded to 3 chapters· "Poliomyelitis," "Poliomyelitis Pathogenesis and Histopathology" and "Poliomyelitis Control" One chapter on a recently identified disease, "Hemorrhagic Fever," has been added

Four chapters included in the second edition are not represented separately in this book Hemagglutination by viruses is included in the chapter on "Virus-Host Cell Relation" Diagnosis of viral and rickettsial infections is considered in the several chapters dealing with specific disease entities Epidemic keratoconjunctivitis is discussed in relation to the viruses which most commonly are associated with the syndrome, i.e., Adenoviruses. Rift Valley fever is described in the chapter on

"Miscellaneous Arthropod-Borne Virus Infections of Man."

Because of the very large number of references, some of those that were chiefly of historical interest and were included in the second edition have been deleted from the reference lists Such references are identified by the absence of parentheses about either the author's name or the year of publication.

This book is designed, as were the previous editions, to provide comprehensive information relative to viral and rickettsial infections which meets the needs of graduate students of biology, including those who are preparing for a career in medicine It is hoped that it will also prove useful to physicians, teachers and investigators in the biologic sciences

The National Foundation has, as with previous editions, generously provided support for the preparation and the publication of this volume Because of this continuing aid it will be possible to distribute the book at a price considerably below that which would normally be required

The editors are deeply grateful to all of the contributors for the large effort they expended on and the care and the thought they gave to the preparation of manuscripts, the selection of references and the development of illustrative material. They are grateful also for the generous spirit of wholehearted co-operation that has characterized this collaborative undertaking throughout the lengthy process of its development

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Contents

out into the cytoplasm Shortly after the obvious increase in S antigen, appreciable amounts of hemagglutinin become discernible in the cytoplasm The two subunits move toward the cell wall where the hemagglutinin is assembled around the S antigen and lipid is incorporated to make the finished infectious particle (cf. Chap. 4 and Figs 34 to 38).

Since the nucleic acids of phage, tobacco mosaic virus and other viruses as well constitute the unadorned infectious principal, they are of great interest not only to virologists but also to biochemists and geneticists. For example, the former see such nucleic acids as tools for studying the production of proteins, i.e , those specific proteins which eventually form the protective cover for the infectious material, as well as proteins which in some instances have enzymatic activity The latter regard them as among the simplest packages capable of carrying genetic information and hope from elucidation of nucleic acid replication and function in viruses to gain insights applicable to genes in higher organisms Incidentally, the viral nucleic acids behave in some respects like the DNA of pneumococci and other bacteria which cause genetic transformation, but perhaps are more aggressive in that they enslave a totally unrelated host cell and usually work it to death in the course of producing quantities of new virus. The bacterial transforming substances, on the other hand, capture closely related organisms and force them to produce progeny like the original organism which supplied the factor

Mutants appear during viral multiplication and are recognized by a variety of distinguishing characteristics Among phages, such markers which are conveniently used in genetic studies are varying plaque types and differing bacterial host ranges, while among influenza viruses antigenic peculiarities, virulence and physical properties provide means for recognizing individuality When infection is induced by two readily distinguishable mutants, most of the progeny resemble one or the other parent, but some individual progeny possess the markers of each parent The last are called recombinants Perhaps even more interesting is the observation that infection with one mutant in the presence of inactivated particles of another mutant can result in a proportion of infectious progeny with certain markers from the inactivated material. This phenomenon is discussed in a number of later chapters, particularly 3, 4 and 7.

The fact that viruses undergo mutations either in nature or in the laboratory is of practical, as well as theoretical, importance Thus, the 17D strain of yellow fever virus, which provides one of the most useful live attenuated vaccines available to preventive medicine, is quite different in many respects from its parent strain (cf. Chap 15) The modern methods for obtaining clones of viruses, particularly the plaque and terminal dilution technics, have made it possible to proceed rather rapidly and with considerable assurance in the search for highly attenuated, reasonably stable mutants of the polioviruses for use in a live vaccine That mutants can be disadvantageous to man is illustrated by influenza (cf. Chap 31) The pandemic of 1957 was caused by the Asian strain of influenza A virus which was appreciably different antigenically from other known strains Furthermore, the vast majority of the world populations had no previous experience with this particular strain and hence were susceptible

PATHOLOGY

Because virology, from its inception, has been concerned with disease, and because most of the early mammalian virologists were trained pathologists, the lesions which characterize the infections have received much attention. The pathologic picture of each disease is described in detail in the appropriate chapters of this book The student probably will be impressed with the monotonous similarity of the lesions found in a given organ system, even though these are caused by different viral or rickettsial agents Thus, the gross and the microscopic findings in pneumonias caused by influenza or psittacosis viruses, or the rickettsia of Q fever, present more common features than differences The same may be said for the viral encephalitides and for the typhus-spotted fever group of rickettsial diseases This should not be surprising since the pathologist requires bacteriologic aid to differentiate most of the bacterial pneumonias from one another. It is of some consequence, however, that viral agents elicit a different tissue response in a given organ than do pathogenic bacteria These differences are found not only in the parenchy-

and antibiotics of therapeutic usefulness in human disease and are presumed to divide by binary fission There is unanimity of opinion that agents of this group are more closely related to rickettsiae than to other viruses, all of which are smaller in size The smallest of the viruses, i e, that causing foot and mouth disease, is almost as minute as the larger protein molecules (cf Fig 1, Chap 2)

As might be anticipated, the larger viruses are more complex structurally, antigenically and biochemically than the smaller ones The elementary bodies of vaccinia, which are the virus particles themselves, appear on electron microscopy as cuboidal objects with dimensions of about 210 × 260 mμ, granules, presumably composed of nucleoprotein, lie in a homogeneous cytoplasm which is surrounded by a membrane The virus particles contain at least 5 different antigens Proteins, lipid, deoxyribonucleic acid (DNA) and copper are constituents of vaccinia virus, while biotin and flavin are probably integral components of the virus (cf. Chap 32). Poliovirus is the best characterized of the small mammalian viruses. It is a spherical particle, almost 25 mμ in diameter, composed of protein and ribonucleic acid (RNA) and containing at least 2 antigens (cf. Chaps 22 to 24).

Probably the most exciting aspects of modern virology are those concerned with the nature of the processes by which new infectious viral particles are formed These processes appear, at first glance at least, to bear little relationship to the classic form of reproduction with the mating of male and female elements, cellular division following pairing of chromosomes and transmission of hereditary characters according to Mendelian laws Neither do they resemble the division of somatic cells which in a simplified form operates in the binary fission of bacteria and rickettsiae. The brilliant investigations dealing with replication of bacteriophages, tobacco mosaic virus, the myxoviruses and others are recounted in a fascinating manner in the several chapters by Hershey, Stanley et al, Hirst and Cohen; the reader is urged to consult these detailed presentations The present discussion will only epitomize a few of the more pertinent developments.

Some of the most imaginative work in biology during the past decade has resulted from the efforts of those studying the bacterial viruses The salient points dealing with replication are as follows: the sperm-shaped bacteriophage attaches itself to the bacterial wall by the tip of its tail, then after digesting away a portion of the wall, it injects its nucleic acid core into the bacterium, leaving the empty protein shell outside the organism. During the few minutes when infectious phage is not detectable in the newly parasitized cell, the phage nucleic acid reduplicates itself manyfold while almost simultaneously the specific phage proteins that make up the coat are produced in abundance. For this process, the phage employs the energy apparatus of the bacterium, which remains functional but substitutes manufacture of viral DNA and protein for creation of new bacterial protein, DNA and RNA Somehow the subunits of phage, i e, the nucleic acid strands and the protein coats, are assembled and result in infectious particles (cf Chaps. 4 and 7).

Tobacco mosaic virus, the filtration of which in the last century introduced the concept of filterable viruses, has continued to provide a model system with which fundamental contributions have been made to biology With this, the first of the viruses to be highly purified, crystallized and shown to consist solely of RNA and protein, it now appears that under proper experimental conditions the nucleic acid alone can infect cells The protein of the virus serves as a protective tube around the fragile nucleic acid filament during its journey from one intracellular environment to another (cf Chap 2, Fig 13)

Influenza viruses and their relatives have provided excellent models for examining the replication process in mammalian viruses These medium-sized viruses, the basic constituents of which are ribose nucleic acid, protein and lipid, contain at least 2 antigens, i e, a nucleoprotein "S" antigen and a protein hemagglutinin. Using the tools of modern virology such as electron microscopy, radioactive compounds and fluorescent antibody, as well as the older technics, it has been possible to trace the events from the time infectious virus enters the cell until other infectious particles emerge. The virus particle disintegrates on reaching the cytoplasm Several hours later, S antigen has increased manyfold in the nucleus and has begun to spread

out into the cytoplasm Shortly after the obvious increase in S antigen, appreciable amounts of hemagglutinin become discernible in the cytoplasm The two subunits move toward the cell wall where the hemagglutinin is assembled around the S antigen and lipid is incorporated to make the finished infectious particle (cf. Chap. 4 and Figs 34 to 38).

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matous cells (see below) but also in the cellular infiltrations. The polymorphonuclear leukocyte is the principal cell in the acute inflammation caused by pyogenic bacteria, while mononuclear cells and lymphocytes characterize the secondary inflammatory reaction of the uncomplicated viral lesion

Rivers pointed out in an earlier edition of this book that the primary responses of the *infected cells in all viral maladies are* hyperplasia alone, hyperplasia followed by necrosis, and necrosis alone Examples of viral lesions in which each of these three primary responses predominate are sarcomas produced in the chicken by the Rous agent, pocks on the chorio-allantoic membrane caused by vaccinia virus, and necrosis of anterior horn cells resulting from infection with poliovirus If action of a virus is not extremely rapid and explosive, and if susceptible cells are capable of multiplication, the primary effect of the active agent is stimulation leading to cellular hyperplasia Following the hyperplasia there is usually destruction or necrosis of the cells, which in turn is attended or followed by a secondary inflammation representing a reaction of the neighboring tissues and of the host The balance between the stimulative and destructive tendencies of a virus determines whether hyperplasia or necrosis is the predominant part of the pathologic picture If the action of a virus is explosive or rapid, or if the susceptible cells are incapable of division and multiplication, then the primary pathologic changes are necrobiosis and lysis of cells.

The capacity of viruses to stimulate hyperplasia of cells has an important bearing on the etiology of benign and malignant tumors Although the viral origin of the chicken sarcoma was demonstrated by Rous in 1911 and subsequent years have seen the addition of a variety of tumors of mammals, birds and poikilothermic vertebrates to the list of those caused by viral agents, the only human tumors now known to be of viral etiology are warts The recent advances in virologic knowledge

cancers.

At one time, inclusion bodies were of great importance in viral work and were used for diagnostic purposes. Negri bodies are still important for this reason, but most of the others have become less significant, because more accurate methods of making diagnoses and identifying viruses have been developed. Investigators studying replication of viruses by means of electron microscopy of thin tissue sections and by fluorescent antibody technics have added to the earlier body of data on inclusions which was obtained by microscopic examination of ordinary histologic material *stained with aniline dyes*. Some of the classic inclusions are found only in the cytoplasm and others only in the nucleus, while a few occur in both the nucleus and the cytoplasm For example, Guarnieri bodies of vaccinia, Bollinger bodies of fowlpox, and Negri bodies of rabies are found only in the cytoplasm, the inclusion bodies of herpes simplex and chickenpox occur only in nuclei, and the inclusions of smallpox and paravaccinia are situated in both the nucleus and the cytoplasm Not only does the location of inclusion bodies vary, but their composition and staining reactions may be quite diverse. A Bollinger body consists of a lipoid membrane derived from the host cell and filled with elementary or Borrel bodies embedded in a matrix Guarnieri bodies consist of altered host-cell material in which are embedded many but not all the elementary or Paschen bodies within the cell An intranuclear inclusion is usually an acidophilic mass occupying most of the nuclear area and is surrounded by a clear zone or halo, the basophilic chromatin of the nucleus marginates on the nuclear membrane Recent electron microscopic studies of cells infected with herpes simplex virus have been interpreted as indicating that a small primary body, about 40 mμ in size, differentiates within the nucleus and acquires a limiting membrane These

Similar studies seem to indicate that the elementary bodies of vaccinia and of fowlpox develop entirely in the cytoplasm of infected cells On the other hand, adenovirus particles develop within the nucleus of infected cells, and groups of these particles often form crystalline structures in the nucleus (cf Chap 2, Fig 15). It is evident that the newer methods have added to knowledge of viral inclusions and cytopathology, but much remains to be done in integration of ultramicroscopic and microscopic data

In the typhus-spotted fever group of rickettsial diseases, the primary pathologic lesions are in the small blood vessels where

rickettsiae multiply in the endothelial cells which undergo proliferative and degenerative changes. Secondary inflammatory infiltrations of mononuclear and lymphocytic cells occur in the adventitia of affected vessels. The scattered localized vascular changes with their resultant lesions in adjacent parenchymatous tissues are found throughout the body but particularly in the heart, the lungs and the brain.

Toxic substances are intimately associated with a number of rickettsial and viral agents. These kill mice within a few hours when concentrated suspensions of the microbial agents are injected. Pathologic examination of such animals reveals little except intense vascular congestion. Physiologic studies, particularly in mice affected by the rickettsial toxins, have shown that the basic lesion is increased permeability of capillaries. This results in a series of changes including perivascular edema, hemoconcentration, and eventually death from hypotensive shock. As yet, the role the toxic materials play in the clinical and pathologic pictures of the viral and rickettsial diseases of man is not known.

IMMUNITY AND RESISTANCE

The basic principles of immunology and serology apply in viral and rickettsial diseases just as they do in other fields of medicine and biology. Broadly speaking, virology has been a consumer rather than a creator of serologic technics and of knowledge regarding fundamentals of immunology. Nevertheless, virology contributed one of the most sensitive serologic procedures, i.e., the neutralization test, before the end of the last century and more recently the hemagglutination-inhibition test which has such wide applicability. Moreover, the first of the successful vaccines, i.e., that against smallpox, was described by Jenner almost a century before filterable viruses were recognized as such. Chapter 10 presents in some detail those aspects of serology which are of particular concern to the student of virology, and the chapters dealing with individual diseases discuss many of the immunologic phenomena which are of special importance in these maladies. The excellent chapter "Defense Mechanisms of the Host" by MacLeod in the companion volume, *Bacterial and Mycotic Infections of Man*, should be consulted for general information on the subject and the role of immune mechanisms in the processes of defense.

The early virologists were preoccupied with susceptibility and resistance of various hosts and tissues to the agents under investigation. This is understandable since these workers were able to recognize the presence of their viral agents only indirectly by means of the obvious lesions they produced. That the subject is still of importance is attested by the fact that each chapter in this book which deals with a specific agent has a section entitled "Experimental Infection, Host Range." However, in recent years it has become increasingly evident that many of the viral agents which were originally regarded as extremely limited in their tropism can multiply in organs where they elicit trivial or nonspecific lesions and can be adapted to growth in a variety of tissues and hosts. Poliomyelitis virus illustrates this, for many years it was classified as a highly neurotropic virus the host range of which was limited to primates. Now it is established that the virus multiplies in a variety of tissues in man and monkey, including those of the pharynx, the intestine and the lymph nodes, moreover, involvement of the central nervous system actually occurs in only a small proportion of persons who become infected. Furthermore, all three types of poliovirus grow profusely in tissue cultures prepared from a number of different kinds of cells, while strains of several types have been adapted to growth in embryonated eggs and mice.

The natural resistance of members of a given animal species to a given viral agent is a poorly understood phenomenon though one of great importance. Such resistance, which is unaccompanied by previous experience with the microbial agent, and hence unassociated with antibody immunity, is affected by age, sex, genetic background, state of nutrition, hormonal balance and other factors. The high degree of susceptibility of newborn mice to fatal infection with the Coxsackie viruses and the great resistance of adult mice to this group of agents provide one example of an effect of increasing age on resistance. The influence of hormonal imbalance on natural resistance is illustrated by the effect of pretreatment with cortisone which renders adult mice susceptible to Coxsackie viruses. The possible role of

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Although the viral origin of the chicken sar*coma was demonstrated by Rous in 1911 and* subsequent years have seen the addition of a variety of tumors of mammals, birds and poikilothermic vertebrates to the list of those caused by viral agents, the only human tumors *now known to be of viral etiology are warts* The recent advances in virologic knowledge about the leukemias of chickens and mice and the discovery of a filterable agent capable of eliciting a number of different kinds of malignant tumors in a single mouse have reactivated a ferment of interest in the search for viruses which may be responsible for certain human cancers.

At one time, inclusion bodies were of great importance in viral work and were used for diagnostic purposes. Negri bodies are still important for this reason, but most of the others have become less significant, because more accurate methods of making diagnoses and identifying viruses have been developed. Investigators studying replication of viruses by means of electron microscopy of thin tissue sections and by fluorescent antibody technics *have added to the earlier body of data on inclusions which was obtained by microscopic* examination of ordinary histologic material stained with aniline dyes Some of the classic inclusions are found only in the cytoplasm and *others only* in the nucleus, while a few occur in both the nucleus and the cytoplasm For example, Guarnieri bodies of vaccinia, Bollinger bodies of fowlpox, and Negri bodies of rabies are found only in the cytoplasm; the inclusion bodies of herpes simplex and chickenpox occur only in nuclei, and the inclusions of smallpox and paravaccinia are situated in both the nucleus and the cytoplasm. Not only does the location of inclusion bodies vary, but their composition and staining reactions may be quite diverse A Bollinger body consists of a lipoid membrane derived from the host cell and filled with elementary or Borrel bodies embedded in a matrix Guarnieri bodies consist of altered host-cell material in which are embedded many but not all the elementary or Paschen bodies within the cell An intranuclear inclusion is usually an acidophilic *mass occupying most of the nuclear area and* is surrounded by a clear zone or halo, the basophilic chromatin of the nucleus marginates on the nuclear membrane Recent electron microscopic studies of cells infected with herpes simplex virus have been interpreted

small particles, on release into the cytoplasm, *acquire a second* surrounding membrane and a diameter of about 120 mμ, the larger particles presumably represent infectious virus Similar studies seem to indicate that the elementary bodies of vaccinia and of fowlpox *develop entirely in* the cytoplasm of infected cells On the other hand, adenovirus particles develop within the nucleus of infected cells, and groups of these particles often form crystalline structures in the nucleus (cf. Chap 2, *Fig 15) It is evident that the newer meth*ods have added to knowledge of viral inclusions and cytopathology, but much remains to be done in integration of ultramicroscopic and microscopic data.

In the typhus-spotted fever group of rickettsial diseases, the primary pathologic lesions are in the small blood vessels where

current attack of labial herpes, or finally it may be shed, as by the typhoid carrier, and contaminate the environment. When the agent and the animal cell exist in a state of mutual tolerance, such as occurs in lysogenic bacteria, the pathogen can be transmitted to daughter cells presumably in the immune animal and certainly in experimentally infected tissue cultures. While the persistence of viral and rickettsial agents in recovered man and animals is undoubtedly of great interest and importance from a number of points of view, including long-term immunity, such a mechanism need not necessarily be evoked to explain the permanent immunity which characterizes many viral diseases.

MacLeod pointed out in 1952 that a number of viral, bacterial and fungal diseases characterized by permanent immunity have incubation periods of a week or more. He suggested that in these diseases the rapid secondary immune response might be one of the factors concerned with the failure to have a second classical attack of the malady. Although infection might occur on re-exposure to the viruses of measles, mumps and other long incubation diseases, the recall immune reaction would be expected to augment resistance during the incubation period and result in suppression of overt disease. He also noted that the recall mechanism would not have time to become operative in diseases with incubation periods of less than 3 days, such as the common cold and influenza which are characterized by repeated attacks. Quite a different approach to an explanation for the persistence of immunity and antibodies has been postulated by a number of workers. This employs a variety of hypotheses to account for the continuing presence in the cell of a model for synthesizing antibody that remains active long after the original antigen has been disposed of. Treffers' chapter "Serology and Immunochemistry" in the companion volume *Bacterial and Mycotic Infections of Man* discusses these ideas which lean heavily on templates, self-replicating enzymes and current hypotheses about synthesis of normal proteins. Irrespective of the explanation, the fact remains that antibodies against certain noninfectious antigens, for example, tetanus toxoid, continue to be detectable in the serum of man for years after a course of immunization; here the possibility of contact with the antigen under natural conditions, as in recurrent epidemics of measles, is negligible. It is evident that persistent immunity can result from several mechanisms and it is likely that more than one is operative in many infections.

In discussing the absence of durable immunity in certain viral and rickettsial diseases, it is necessary to recall that the clinical manifestations which characterize the maladies may be caused by a group of related but antigenically dissimilar agents. Detailed information on the types and the strains of pathogens associated with the common cold, influenza, poliomyelitis, Coxsackie infections, dengue and scrub typhus is given in the chapters which deal with these diseases. In some instances the several agents are so dissimilar that infection with one elicits essentially no immunity to another even under experimental conditions, for example, influenza A and C or polio Types I and III. In other instances, notably in dengue and in scrub typhus, the distinguishable strains or types of the etiologic agents elicit complete resistance in man to the homologous pathogen for at least several years but to the heterologous members of the group for only a month or so. In the last two diseases, inoculation of recovered volunteers with heterologous strains after 3 months produces an abortive or atypical illness, but after a year results in a second episode of the classical disease.

The original infection of man with certain viruses almost invariably results in the classical features of the disease, smallpox, measles, chickenpox and rabies provide illustrative examples of this. In contrast, infection with certain other viruses rarely produces the textbook description of the full-blown malady. Thus only a small proportion of persons infected with poliovirus develop paralytic disease, and few persons who become infected with St. Louis, western, or Japanese encephalitis virus display signs of encephalitis. Nonparalytic poliomyelitis or aseptic meningitis occurs in some people infected with the viruses just mentioned, but the majority suffer a subclinical or brief, poorly defined illness. The active immunity which develops in persons following such subclinical or mild infections differs in no way from that found in patients convalescent from severe illness.

properdin and other similar humoral factors in natural resistance of animals to viral infections deserves careful study.

The interference phenomenon is concerned with a cell, animal, or plant which on exposure to viral material promptly develops resistance to infection by virus. The reaction itself is unrelated to classic immunologic responses, although it impinges on and interdigitates with immunologic reactions as is evident from certain of the examples of interference which are cited below. Lysogenic bacteria, i.e., those latently infected with a temperate phage which are capable of growing and multiplying, are resistant to destruction by virulent strains of the homologous phage even though fully susceptible to virulent strains of other phages. Along similar lines, cells treated with noninfectious influenza virus promptly lose susceptibility to active homologous virus. The interference phenomena also extend to the intact animal. Thus, monkeys inoculated peripherally with nonlethal amounts of a neurotropic strain of yellow fever virus will, within a few hours, resist a fatal dose of viscerotropic yellow fever virus. Interference is not limited to homologous or even closely related viruses. For example, monkeys infected with the virus of lymphocytic choriomeningitis are for a short time less susceptible than normal animals to the destructive effects of poliovirus, and, similarly, mice injected intracerebrally with influenza virus resist lethal amounts of the virus of western equine encephalomyelitis. The mechanism of interference is poorly understood. Although there has been hope that the phenomenon might be applicable to measures for the prevention of human disease, this has not yet been realized. However, interference may be of some importance in nature in the geographic limitation of certain arthropod-borne infections. The viruses of yellow fever and dengue interfere with one another in their common vector *Aedes aegypti*; since infection persists for life in such mosquitoes, a naturally infected Aedes presumably would be incapable of transmitting more than one of the agents (cf. Chap. 5).

Active immunity is the state of resistance engendered by a spontaneous attack of an infectious disease, whether classic, atypical or subclinical in its manifestations, by the experimental or intentional production of the disease or a modified form of it or by the injection of a vaccine. In a number of viral and rickettsial diseases, just as in some bacterial and fungal diseases, one attack almost invariably confers lifelong immunity; examples of this are smallpox, measles, mumps, poliomyelitis, yellow fever and epidemic typhus. Most persons recovered from the maladies just mentioned continue to have demonstrable specific circulating antibodies for many years after an attack. Since certain of these diseases, i.e., measles, mumps and polio, are present at frequent intervals in a community, one might account for the persistent immunity and continual presence of humoral antibodies on the basis of repeated contacts with the viral agents and several subclinical attacks. On the other hand, this explanation is inadequate to account for persistent antibodies in persons who recover from yellow fever or typhus and then live many years in the U.S.A. or other areas where these diseases are not endemic. It is often assumed in such instances that the agent persists in the recovered individual just as it does in the typhoid carrier. The best clinical example of such persistence is Brill-Zinsser disease which is a recurrent form of epidemic typhus developing long after the initial attack (cf. Chap. 42). Sufficient instances in which viruses have been recovered from immune hosts have been recorded to show that it is not an unusual occurrence, for example, the demonstration of herpes simplex virus in the gasserian ganglion, of adenoviruses in tonsillar tissue and salivary gland virus in the urine of children many months after initial infection, as well as the finding of psittacosis virus in birds and man, infectious anemia virus in horses and lymphocytic choriomeningitis virus in mice, long after apparent recovery.

The mechanism by which these pathogens survive in the immune hosts is not understood. They exist intracellularly, where they are protected from the usual humoral and phagocytic defense mechanisms of the animal, and presumably maintain a state of parity in a cold war with the cell. When the pathogen gains supremacy and the infected cell is destroyed, the liberated agent may fall prey to the defenses of the host and incidentally stimulate its immunologic mechanism, or it may induce Brill-Zinsser disease or a re-

current attack of labial herpes, or finally it may be shed, as by the typhoid carrier, and contaminate the environment. When the agent and the animal cell exist in a state of mutual tolerance, such as occurs in lysogenic bacteria, the pathogen can be transmitted to daughter cells presumably in the immune animal and certainly in experimentally infected tissue cultures. While the persistence of viral and rickettsial agents in recovered man and animals is undoubtedly of great interest and importance from a number of points of view, including long-term immunity, such a mechanism need not necessarily be evoked to explain the permanent immunity which characterizes many viral diseases.

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Associated in most instances with the development of active immunity against viruses in recovered persons is the appearance of circulating antibodies What relation do such antibodies have to immunity against virus diseases? Animals may possess agglutinins, precipitins and complement-fixing antibodies against certain viruses without being resistant to infection In most instances, but not all, the presence in an animal's serum of neutralizing antibodies against a virus indicates that such an animal is resistant to the active agent. However, some animals recovered from a virus infection are resistant to reinfection without possessing demonstrable neutralizing antibodies.

Passive immunity is a state of resistance to infection produced in a normal person by the parenteral administration of serum containing antibodies, such sera are obtained from actively immunized persons or animals So far as is known, this state of resistance to virus diseases can be brought about only by the injection of serum containing neutralizing antibodies. In passive immunity it is quite obvious that humoral antibodies are the important factors rather than tissue immunity Whether the antibodies introduced into people protect susceptible cells against the entry of virus, whether they act directly on the virus in such a manner as to prevent the production of disease, or, finally, whether they enhance the destruction of virus by certain phagocytic cells, is not known Passive immunity is of great practical interest because of its use in connection with prophylaxis and treatment of virus diseases, a matter discussed later in this chapter

Immunologic principles derived from studies on skin grafting which deal with acquired tolerance may have applicability in certain aspects of viral infections Such tolerance has been defined as an induced state of nonreactivity toward a substance that is normally antigenic—due to a primary failure of the machinery of the immunologic response. Contrary to the usual experience, adult animals will accept skin grafts from distant relatives if prepared by an inoculation of similar tissue given before the immunologic response comes into being at about the time of birth Mice which survive intra-uterine infection with the virus of lymphocytic choriomeningitis appear healthy but have viremia for months after birth without developing an antibody response. On the other hand, mice infected as adults behave in the customary manner developing antibodies after a period of systemic involvement (cf Chap. 46) It has been suggested that the principle of acquired tolerance might be considered in explaining the paradox just described The possibility has not been probed that a similar mechanism is operative in the prolonged viremias that occur in human beings and horses infected with the viruses of serum hepatitis and infectious anemia, respectively

TRANSMISSION

The viruses which cause most of the world-wide epidemic diseases of man have as their sole natural host man himself, hence, human beings serve as the reservoir for the agents Transmission of diseases such as measles, smallpox, poliomyelitis and other members of this group is usually by contact with infected persons (direct) or with their immediate environment which has been contaminated (indirect) Transmission by droplet spread is perhaps best considered as a form of contact infection since it involves reasonably close association between the patient who sneezes or coughs and the susceptible person The source of infectious materials in the measles patient is secretion from the nose and the throat and transmission of this highly communicable disease is by droplet spread and direct contact. In smallpox, the lesions of the skin and mucous membranes, as well as respiratory discharges, are sources of virus for spread by contact, by aerial transmission and by contaminated articles (fomites). Poliovirus appears in the feces and the pharyngeal secretions of infected persons and is transferred directly from hand to mouth and by droplets Details regarding transmission of these and other diseases with similar modes of spread can be found in appropriate chapters in this book

Many of the viral and most of the rickettsial diseases are transmitted to man by arthropods (mosquitoes, lice, fleas, mites, ticks and sandflies). However, in only a few of these does man serve as the reservoir. Urban yellow fever, in contrast with jungle yellow fever,

and epidemic typhus are examples in which the maladies are spread from man to man by mosquitoes and lice, respectively. Certain of the human diseases in which arthropods serve as vectors are maintained in nature by infection chains which involve wild animals or birds as principal hosts and reservoirs. Moreover, the arthropod vector also serves as one of the reservoirs in some instances, for example, in scrub typhus and spotted fever where the rickettsiae are passed transovarially in the mite and the tick, respectively, from one generation to the next. In these cycles in nature, man is only an accidental participant. Indeed, when infected he usually does not reinfect vectors and perpetuate the transmission; illustrative examples are Colorado tick fever and murine typhus as well as the two rickettsial diseases mentioned in the preceding sentence. On the other hand, the individual who contracts yellow fever in the jungle from the bite of a Haemagogus mosquito that acquired the virus from an infected monkey can, on becoming ill with viremia in a community infested with *Aedes aegypti*, start an epidemic of urban yellow fever which will spread as long as susceptibles are available and Aedes persist. The ecology and the epidemiology of the vector-transmitted diseases are frequently complicated and sometimes unique, the appropriate chapters should be consulted for details. Two of the most complex cycles are worthy of mention even though they are not directly concerned with human disease. The virus of swine influenza has the lung worm as an intermediate host, this parasite itself has a complicated cycle and its eggs, on excretion in swine feces, are taken up by earthworms where they develop through several larval stages. The earthworms are in turn eaten by pigs, after which the developing lung worm larvae migrate to the lungs. Throughout the lung worm sojourn the virus remains masked but under appropriate conditions it is released by the parasite in the lung, producing swine influenza. The rickettsialike organism causing the highly fatal salmon poisoning disease of dogs has as an intermediate host an intestinal fluke which in turn has a complex cycle requiring snails, salmonid fish and mammals for maturation, canines develop the disease after eating infected fish.

Very few viral diseases are water-borne, infectious hepatitis being an outstanding example (and even this is not usually spread by water but by contact). Only a few viruses are spread by food, several milk-borne epidemics of poliomyelitis and at least one of infectious hepatitis have been reported. One virus disease, rabies, is ordinarily transmitted only by the bite of a rabid animal.

PREVENTION AND TREATMENT

Three of the 6 human diseases subject to international quarantine regulations are caused by viral or rickettsial agents; these 3 are smallpox, yellow fever and epidemic typhus (the other 3 are plague, cholera and relapsing fever). Although quarantine measures have become highly efficient in limiting the international spread of these ancient pestilences, it is worthy of note that the procedures in each instance go far beyond temporary limitation of freedom of affected and exposed persons. They include, in addition, enforcement of sanitary practices, disinfestation or decontamination, and, in a number of instances, vaccination of susceptibles. On the other hand, because of lack of effectiveness in controlling spread of disease in local communities, quarantine has been abandoned or modified for patients, and their known contacts, with measles, rubella, chickenpox, mumps, poliomyelitis and most other viral infections in which man is the reservoir of the agent.

Immunization procedures provide a shield for the individual and the community against a number of viral and rickettsial diseases. Two vaccines, each containing live attenuated viruses, are among the most important weapons of public health officers, these are smallpox and yellow fever vaccines. When properly used, they are safe, they protect almost every individual, and they promptly stop an epidemic. Three other vaccines which are highly effective, but not so nearly perfect as the two just mentioned, are those against epidemic typhus, influenza and poliomyelitis. Each of these three consists of noninfectious antigen, and each has been administered to millions of persons. In addition, there are vaccines which have an appreciable value but have been employed less widely. In some instances the limited use is dependent on the restricted group of persons at risk, for example, adeno-

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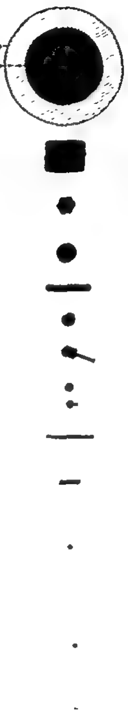
FIG. 1 APPROXIMATE SIZES OF VIRUSES AND *Reference Objects* [*]

	Diameter or width × length in mμ
Red blood cells	7500
B. prodigiosus (Serratia marcescens)	750
Rickettsia	475
Psittacosis	270[†]
Myxoma	250 × 290
Vaccinia	210 × 260
Pleuropneumonia organism	150
Herpes simplex	130
Cytoplasmic virus (*Tipula paludosa*)	130[†]
Rabies fixe	125
Newcastle disease	115
Avian leukosis	120
Vesicular stomatitis	65 × 165
Polyhedral virus (*Bombyx mori*)	40 × 280
Influenza	85[†]
Adeno	75[†]
Fowl plague	70
[illegible] *E. coli* bacteriophage	65 × 95
Chicken tumor I (Rous sarcoma)	65
Equine encephalomyelitis	50
[illegible] *E. coli* bacteriophage	45
Rabbit papilloma (Shope)	45[†]
Tobacco mosaic and strains	15 × 300
Cymbidium (orchid) mosaic	12 × 480
Genetic unit (Muller's est. of max. size)	20 × 125
Southern bean mosaic	30
Tomato bushy stunt	30[†]
Coxsackie	27
Poliomyelitis	27[†]
Turnip yellow mosaic	26[†]
Tobacco ringspot	26[†]
Yellow fever	22
Squash mosaic	22[†]
Hemocyanin molecule (Busycon)	22
Foot-and-mouth disease	21[†]
Japanese B encephalitis	18
Tobacco necrosis	16
Hemoglobin molecule (Horse)	3 × 15
Egg albumin molecule	2.5 × 10



[*] Revised in 1958 by R. C. Williams from Stanley, W. M., 1947, Chemical studies on viruses, Chem. & Eng. News 25, 3786-3791.
[†] Diameter obtained from frozen dried specimens.

tary bodies were as large as certain bacteria and hence were regarded as small living organisms. Then Elford, 1931, began his important work on the filtration of virus preparations through graded collodion membranes of known porosity. He soon established the fact that different viruses possessed different and characteristic sizes and that some viruses possessed diameters of about 10 mμ, while others had diameters of about 300 mμ This work on a physical property, namely size, had a profound influence on the thinking of the times, for investigators began to wonder about the true nature of a structure only 10 mμ in diameter which possessed the ability to replicate or to bring about its replication .

Studies on the chemical properties of viruses also began to assume importance around 1930 Vinson and Petre (1929, 1931) showed that tobacco mosaic virus could be subjected to numerous chemical manipulations with retention of virus activity Many investigators working with different viruses conducted studies on the effect of different chemicals on virus activity Chemical investigations directed toward the concentration and the purification of certain viruses were started One of these led to the isolation, in 1935, of tobacco mosaic virus in the form of a crystalline material of unusually high molecular weight (Stanley, 1935) This material was subsequently shown to be a nucleoprotein with a particle size of

lowed by the isolation of over a dozen viruses in highly purified, and in some instances, crystalline form Recently, poliomyelitis virus (Schaffer and Schwerdt, 1955), Coxsackie virus (Mattern and du Buy, 1956) and an insect virus (Williams and Smith, 1957) were obtained in crystalline form All these purified viruses have been found to be at least as complex as a nucleoprotein with some containing ribose nucleic acid and others deoxyribose nucleic acid. A recent finding of the greatest importance is the discovery by Fraenkel-Conrat (1956), and independently by Gierer and Schramm (1956), that a nucleic acid preparation possessing virus activity can be obtained from tobacco mosaic virus nucleoprotein.

A chart showing the approximate sizes of several viruses and some reference materials, prepared largely from data obtained with the electron microscope, is provided in Figure 1.

PURIFICATION

Chemical Methods of Purification

The purification of a virus by chemical technics involves the use of a procedure or, usually, of a series of different procedures which results in a relative enrichment of virus as measured by biologic assay. It is advisable to determine the pH stability range of the virus under investigation, for chemical treatments involving hydrogen ion concentrations outside of the range of stability of the virus cannot be employed. Another factor of considerable importance is the selection of the starting material. Thus, for example, other factors, such as approximate amount of virus, etc., being equal, it would be better to use the extra-embryonic fluids of the infected chicken embryo rather than an extract of emulsified whole chicken embryo as starting material, for the former contains far less extraneous material than does the latter. Every effort should be made to secure a starting material containing the highest possible concentration of virus and the lowest possible concentration of nonviral materials having properties similar to those of the virus Because viruses differ in their chemical properties, and because the impurities associated with any given virus also differ, it is not possible to outline a definite procedure for the purification of viruses However, because all purified viruses now known are at least as complex as a nucleoprotein, the general methods which have been employed in work with proteins have been found to be valuable in work with viruses Some degree of purification usually can be achieved by treatment with methyl, ethyl or butyl alcohol, or with salts such as ammonium or magnesium sulfate. Generally, increasing amounts of alcohol or salt are added to aliquots of a virus preparation buffered to a definite hydrogen-ion concentration, and the amount of alcohol or salt necessary to precipitate all of the virus activity is determined Any impurities remaining in the supernatant liquid can then be separated from the virus Another general procedure of value involves the adsorption of virus on some material at a given pH or temperature and the separation of this material, plus adsorbed virus, from the bulk of the liquid and accompanying impurities, followed by the elution of the virus from the adsorbent

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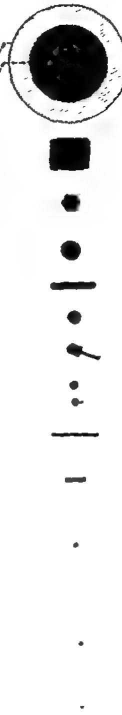


* Revised in 1958 by R. C. Williams from Stanley, W. M., 1947, Chemical studies on viruses, Chem. & Eng. News 25, 3786-3791.

† Diameter obtained from frozen-dried specimens.

at another pH or temperature The adsorption of tobacco mosaic virus on celite at pH 4.5 followed by elution at pH 7 (Stanley, 1935), or the adsorption of influenza virus on chicken red blood cells at 4° C (Hirst, 1941; McClelland and Hare, 1941) are examples of the use of this procedure

Different enzymes have been used to digest protein impurities present in virus preparations Of course, it is necessary to demonstrate that the enzyme used does not cause inactivation of the virus It is also necessary to develop a method for separating the enzyme, as well as the digestion products, from the virus Occasionally, iso-electric precipitation, either of the virus or of protein impurities, can be used to good advantage Certain viruses have also been purified by use of immunochemical methods An antiserum to a crude virus preparation is made and then absorbed with extracts of normal materials from the same kind of host used for production of the virus The residual antibodies presumably are for the virus, and from the virus-antibody precipitate the virus can be obtained by dissociation or in some cases by removal of the antibody portion by means of enzymatic digestion

Physical Methods of Purification

When chemical methods were used in attempts to purify certain viruses which were less stable than tobacco mosaic virus, the viruses were largely inactivated Consequently, physical methods of purification have been explored extensively, particularly high-speed centrifugation

Vacuum-Type Angle C[illegible]

[illegible] purify vaccinia and fowlpox viruses, it did not gain immed[illegible]

[illegible] knowledge concerning the sizes of various viruses. However, when the approximate sizes of several viruses became known from filtration experiments, it was apparent that viruses could be sedimented readily by centrifugal fields of from 50,000 to 100,000 times gravity

Although the first ultracentrifuges were air driven, high-speed electric drives capable of operating at 50,000 r p m. or more have been developed within the past few years. Suitable bearings for the connection between drive and rotor and for the support of large rotors operated in a vacuum chamber have also been developed so that these high-speed drives have largely replaced the air turbine.

The rotor is so designed that, while rotating in a high vacuum, its contents are at atmospheric pressure. Since the rotor spins in a vacuum, there is little friction, and very little heat is generated For use with unstable viruses, the rotor may be cooled to about 0° C., and the material may be kept cold during the entire centrifugation process

Fortunately, in the cases of several viruses, starting materials have been found in which the impurities have rates of sedimentation quite different from that of the virus. In these cases the starting material is first centrifuged at a speed and for a length of time sufficient to sediment practically all materials which migrate more rapidly than the virus The supernatant liquid is then removed and centrifuged at a speed and for a length of time necessary to sediment all or most of the virus The supernatant liquid is discarded, and the pellets of virus, which are usually small and opalescent, are suspended in a liquid known to provide a favorable environment. Subsequent cycles of differential centrifugation are then employed until tests indicate that practically all light and heavy impurities have been removed

The method of differential centrifugation has been used to purify the viruses of tobacco mosaic (Wyckoff et al , 1937) potato latent [illegible]

[illegible] (Ross, 1941a), equine encephalomyelitis (Taylor et al , 1943a), influenza (Taylor et al , 1943b, Stanley, 1944a, Sharp et et , 1944b), Lansing poliomyelitis (Loring and Schwerdt, 1946, Schaffer and Schwerdt, 1955), Southern bean mosaic (Price, 1946), Newcastle disease (Bang, 1946, Cunha et al , 1947), polyhedral disease of silkworm (Bergold, 1947), potato yellow-dwarf (Brakke et al , 1951), wound tumor (Brakke et al, 1954), Coxsackie (Mattern and du Buy, 1956), avian myeloblastic leukosis (Beard, 1956), avian erythroblastosis (Beard et al , 1957) and *T paludosa* [illegible]

as [illegible]

And [illegible] of Japanese B encephalitis in mouse brains (Duffy and Stanley, 1945), considerable difficulty was encountered, for the extracts of the

FIG. 2. Crystals of tobacco-mosaic virus (×675) (Stanley, W. M., 1937. Crystalline tobacco-mosaic virus protein, Am. J. Botany *24*, 59-68)

normal host were found to contain material which sedimented at a rate near that of the virus. If it is assumed that materials of the normal hosts will also be present in the extracts from the diseased hosts, it is obvious that such impurities could never be separated from the viruses by means of differential centrifugation. Other means, such as immunochemical procedures, must be employed to separate impurities of this type.

It should be emphasized that purification by means of differential centrifugation is especially suitable for unstable viruses, since the operations can be conducted quickly and in the cold (Stanley and Wyckoff, 1937). It seems likely that this method will continue to be used [illegible]

The main difficulty encountered has been a certain amount of stirring within the centrifuge tubes due to convection currents resulting in a small proportion of the virus remaining in the supernatant liquid. This difficulty usually is not serious in connection with purification procedures, since only a small percentage of the virus remains in the supernatant liquid. Of [illegible]

experimentation with continuous-flow types of centrifuge, since these have a considerably larger capacity. Stanley (1942a) reported that large amounts of purified tobacco mosaic virus could be prepared by means of the commercially available Sharples Laboratory Supercentrifuge equipped with the regular clarifier bowl operated at a speed of 50,000 r.p.m. by means of compressed air. Stanley (1944a) and later Taylor et al. (1945) demonstrated that influenza virus in the extra-embryonic fluids of infected chick embryos could be purified by means of the Sharples centrifuge. In the cases of the PR8 and Lee strains, about 85 to 90 per cent of the virus could be recovered in the sediment in the bowl at rates of flow around 50 cc. per minute. Because of the effi-

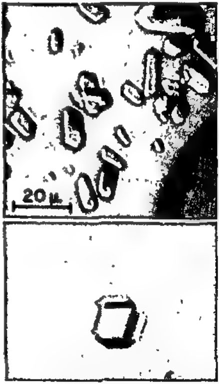


FIG. 3. (*Top*) Crystals of MEF-1 poliomyelitis virus. (Schaffer, F. L. and [illegible]

phys. acta *28*, 241-246)

ciency and the ease of this process, and because of the purity of the product, this method has been adopted for the commercial production of purified influenza virus for use in vaccines (Stanley, 1945). The method has been employed by Randall et al (1947) for the purification of Japanese B encephalitis virus Cox et al (1947) found that alcohol precipitation, followed by continuous-flow centrifugation, provides an efficient method for working with very large volumes of extra-embryonic fluid in the production of influenza vaccine

During recent years a combination of chemical and physical procedures has been used quite successfully for the purification of several viruses These have yielded crystalline preparations of turnip yellow mosaic virus (Markham and Smith, 1949, Cosentino et al, 1956), tobacco ring spot virus (Steere, 1956), poliomyelitis viruses (Schaffer and Schwerdt, 1955, Schwerdt and Schaffer, 1956) and Coxsackie virus (Mattern and du Buy, 1956) Crystals of tobacco mosaic, the first virus to be obtained in crystalline form, are shown in Figure 2, and crystals of poliomyelitis virus, the first animal virus to be obtained in crystalline form, are shown in Figure 3.

IDENTIFICATION OF VIRUS WITH PURIFIED PRODUCTS

Chemical Fractionation

When a supposedly purified product has been obtained, it is necessary to prove that the material in question actually consists of virus and not of a mixture of virus and extraneous materials One way of doing this is to subject the purified product to a series of chemical procedures which may result in the separation of the material into two or more fractions. Then these fractions can be tested for their specific virus activity, that is, activity per unit weight, and if the specific virus activity of one fraction is greater than that of other fractions, the results provide definite evidence that the original material actually consisted of a mixture of virus and impurities. However, if all fractions are found to possess the same specific virus activity, the results can be regarded as providing evidence that the purified product is actually the virus It should be emphasized that a series of different types of chemical fractionation procedure should be used, for although a virus and impurity might be fractionated in the same proportion by one procedure, it is highly unlikely that they always would be fractionated in the same proportion by a wide variety of procedures However, failure to secure fractionation of a purified product is only negative evidence, and such tests gain weight only as they are increased in number and in variety (Stanley, 1938a).

One simple method of securing the separation of a purified virus product into two fractions consists of the addition of sufficient ammonium sulfate or magnesium sulfate to cause the precipitation of only part of the purified product. The precipitated material can be separated from the liquid portion by centrifugation or filtration, and the specific virus activity of the two fractions can be determined. This procedure was used in connection with early tests on tobacco mosaic virus, and it was found that the specific virus activity of the two fractions was the same It may be of interest to note that when purified preparations of two strains of tobacco mosaic virus were mixed and subjected to fractionation with salt, an almost complete separation of the strains was accomplished.

It is obvious that other methods of fractionation such as the use of alcohol, acetone, lead acetate or specific antisera, as well as isoelectric precipitation, can be used Another procedure of importance consists of treating the purified product with an adsorbent, such as charcoal, celite or aluminum hydroxide, followed by tests on the adsorbed and the unadsorbed portions of the preparation. In all cases, it is necessary to prove that the procedure used to achieve fractionation does not cause inactivation of the virus However, an important method of testing for homogeneity consists in the deliberate destruction or inactivation of a portion of the purified product, followed by tests for specific virus activity of the remainder. Reagents such as acids, alkalis, urea, enzymes and various detergents, as well as heat or high pressures, can be used for this purpose If the specific virus activity of the remaining portion is the same as that of the starting material, the results can be regarded as providing evidence of homogen-

partial inactivation of the virus or to the preferential destruction of virus in a mixture of virus and impurity

Advantage should be taken of special situations which provide opportunities for fractionation Thus, in the case of influenza virus, adsorption of virus on washed chicken red blood cells provides a means of fractionation With impure preparations of influenza virus, adsorption on chicken red blood cells followed by elution yields a product of higher virus activity However, with highly purified influenza virus preparations the virus activity is unchanged following adsorption on and elution from chicken red blood cells

Physical Fractionation

Since physical methods are in general, somewhat milder than chemical methods, they have been used widely in attempts to fractionate virus preparations The general approach has been similar to that described above for chemical methods The purified virus preparation is subjected to one or more physical processes which result in the separation of the material into two or more fractions, and the specific virus activities of these are then determined Fractionation has been achieved by means of centrifugation electrophoresis, chromatography and filtration through collodion membranes In early work with purified tobacco mosaic virus, Stanley (1937b) centrifuged solutions of the virus at pH 2 4, 6 7 and 9 4, so that about 85 to 95 per cent of the protein was removed from the upper portions of the supernatant liquids, he found that the specific virus activity of the separated upper and lower portions of the solutions was the same Since the iso-electric point of this virus is about pH 3 5, the virus was sedimented on both sides of the iso-electric point, that is, as negatively charged particles and as positively charged particles The results provided a powerful argument against the idea that virus activity is due to an entity adsorbed on the protein or to a dissociable active group attached to the protein

In contrast with centrifugation, which permits fractionation by virtue of movement in a centrifugal field, electrophoresis achieves the same result through movement in an electrical field. If the pH range of stability of a given virus is sufficiently broad, the virus can be caused to migrate as either negatively or positively charged particles If, following movement over appreciable distances and under different conditions of pH, the specific virus activity of different portions of the liquid remains unchanged, it may be concluded that the preparation is homogeneous with respect to electrophoretic mobility Since it is highly unlikely that the virus and an impurity would migrate at exactly the same rate at different pH's, such results can be regarded as strong evidence that the virus preparation is pure Tests of this nature have been made with several virus preparations, including those of tobacco mosaic (Eriksson-Quensel and Svedberg, 1936), rabbit papilloma (Sharp et al, 1942) and influenza (Miller et al, 1944)

Although the sizes of practically all viruses for which there are suitable biologic tests have been estimated by means of ultrafiltration, this technic has not been used extensively for the fractionation of purified virus preparations Whenever the technic has been used, however, it has generally proved to be satisfactory Collodion filters which will just permit the virus to pass or will just retain the virus are used to determine whether or not the preparation contains impurities possessing filtration characteristics different from that of the virus As in the cases of centrifugation and electrophoresis, virus preparations at different hydrogen-ion concentrations can be tested If the material passes through or is retained by filters under the same conditions under which the virus activity passes through or is retained, the results provide evidence that the material in question is actually the virus

CHARACTERIZATION OF VIRUS MATERIALS

Ultrafiltration

The oldest physical procedure employed in the study of viruses is filtration Bacteriologists had learned early that bacteria could be retained by certain types of filters Iwanowski, 1892, observed that the agent responsible for the tobacco mosaic disease was not retained by a filter which would hold back all pathogenic bacteria then known Later in the study of virus diseases, it was found that filters could be produced which would retain viruses

Allard, 1916, found that, even though tobacco mosaic virus would pass through a Berkefeld filter, it was held back by a Livingstone atometer porous cup Since that time, filters graded for pore size have been made available, and these can be used for the determination of the approximate sizes of viruses, Elford, 1931, Ferry, 1936.

In order to determine the size of a virus particle by means of ultrafiltration, one attempts to pass it through a series of filters, graded with respect to pore size Then the size of the virus is related to the size of the pores in the finest filter through which the particles will pass Several complications are encountered in filtration It has been found necessary to use surface tension active substances, such as sodium oleate or nutrient broth, in order to prevent clogging of the filters Even when this is done, some materials known to be smaller than the pores will not pass This is thought to be due, at least in part, to the surface electric charges of the filter and the particles. Elford has shown experimentally that there is no exact correspondence between the average pore size and the size of the particles By passing colloidal particles with dimensions determined by other means through a graded series of filters, Elford (1933) obtained the data presented in Table 1 showing the approximate relationship between pore size and particle size. It can be seen that, for fine filters, the average pore size must be from 2 to 3 times the particle diameter in order for the particles to pass. For filters with large pores there is more nearly exact correspondence between average pore size and the size of the particles which will pass

TABLE 1 THE RELATIONSHIP BETWEEN THE AVERAGE MEMBRANE PORE DIAMETER AND THE RATIO $\frac{\text{PARTICLE DIAMETER}}{\text{PORE DIAMETER}}$

AVERAGE MEMBRANE PORE DIAMETER IN mμ	$\frac{\text{PARTICLE DIAMETER}}{\text{PORE DIAMETER}}$
10- 100	⅓- ½
100- 500	½- ¾
500-1,000	¾-1

Filtration has been applied to the determination of the particle size of many viruses Some of the sizes indicated in Figure 1 were estimated by this method. The precision of the method can be evaluated by comparing sizes calculated from results of filtration with those obtained by other means. Elford and Andrewes (1932) filtered vaccinia virus and showed that it would just pass through a filter with an average pore diameter of 250 mμ One can estimate, by using the correction factor shown in Table 1, that the diameter of the virus is between 125 and 175 mμ It has since been shown by means of the ultracentrifuge (Elford and Andrewes, 1936; Smadel et al., 1938) and the electron microscope (Green et al, 1942) that the diameter of vaccinia virus is about 225 mμ. The diameter of influenza virus was determined by filtration (Elford et al, 1936) to be between 80 and 120 mμ, whereas by means of the electron microscope the diameter was found to be 82 mμ (Williams, 1953). These data are sufficient to indicate that the method of ultrafiltration can be used to obtain an estimate of the size of a virus but it is apparent that no high degree of precision can be expected However, filtration does have a special use in those cases where the purity of the virus preparation is not great enough to permit other methods to be used

DIFFUSION

One of the important problems confronting those who study viruses is the determination of the size and the shape of the virus particles It has already been shown how ultrafiltration can be used to obtain some idea of size. It is obvious that the electron microscope is admirably adapted to this end. However, until the electron microscope was developed, it was necessary to rely upon indirect methods of [illegible]

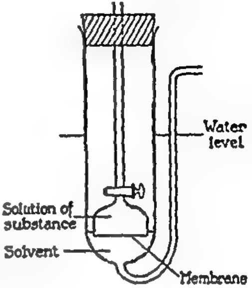
formation which cannot be obtained with the electron microscope. The methods, which include diffusion, ultracentrifugation, viscosity and stream double refraction, stand out for their utility. All of these involve the movement of a particle with respect to the medium in which it is suspended. From the way in which particles move in their surrounding

medium, it is possible to determine something of their size and shape It is the purpose of authors in the following paragraphs to indi- [illegible]

[illegible] particle meets resistance in such movement, and this resistance is proportional to its friction coefficient Thus, in order to interpret diffusion and sedimentation results, it is necessary to understand something about this coefficient

When a particle such as a virus moves through a medium such as water, its motion is opposed by frictional resistance The frictional force, F, acting on the particle is directly proportional to its velocity, v The proportionality constant is called the friction coefficient, f. Therefore, $F = fv$. The size of f depends upon the viscosity of the liquid and upon the size and the shape of the moving particle. According to Stokes' law, $f = 6\pi r\eta$ for a spherical particle where r is the radius and η is the viscosity The viscosity of the liquid can be measured directly Thus one can calculate the radius of a particle from a knowledge of its coefficient of friction If the particle is not spherical, it is more difficult to interpret the meaning of f In this case, the coefficient is an intricate function of the various dimensions of the particle and of the viscosity of the liquid

It is commonly known that if a layer of water is placed very carefully over a concentrated solution of any material the solute molecules will diffuse into the water layer The rate at which a particle diffuses is proportional to the concentration gradient and to a characteristic of the particle known as the diffusion coefficient, D. According to Fick's law, $dS = -DQ\frac{dc}{dx}\,dt$, where dS is the amount of material which will diffuse across an imaginary plane of area, Q, in time, dt, when the concentration gradient is dc/dx Independently, Einstein and Sutherland came to the conclusion that the diffusion coefficient of a particle is inversely proportional to its friction coefficient $D = \frac{RT}{Nf}$, where R is the gas constant, T is the absolute temperature, and N is Avogadro's number Thus, if the



[illegible]

diffusion coefficient of a material can be measured, f can be calculated directly

There are two generally used methods for measuring the diffusion coefficient. The simpler involves placing a solution of the material in a vessel to which a thin, porous membrane has been sealed (Northrop and Anson, 1929) A diagrammatic representation of such a diffusion cup is shown in Figure 4 This filled cell is then placed in contact with pure solvent Through the process of diffusion, the particles on the inside of the cup gradually pass out through the porous membrane into the solvent The diffusion coefficient can be evaluated by measuring the ratio of the concentrations of diffusible material inside the porous cup and outside after a given period of time

A second commonly used method is to place a solution of diffusible material in direct contact with a layer of solvent Several sorts of apparatus have been designed to accomplish this (Lamm, 1937, Neurath, 1942, Kahn and Polson, 1947) After the initial boundary between the diffusing material and the solvent is established, it will gradually become broad because of diffusion, resulting in a gradual change in concentration between the solution

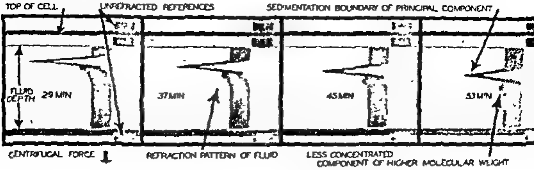


FIG 5. Ultracentrifuge diagram of a 2-component system

and the solvent. This gradual change in concentration can be determined by various optical methods, and from its exact nature it is possible to calculate the diffusion coefficient of the material Neurath and Saum (1938) first attempted the measurement of the diffusion coefficient of tobacco mosaic virus Later, Neurath and Cooper (1940) used it to measure the diffusion coefficient of tomato bushy stunt virus Lauffer (1944a) and Schramm and Bergold (1947) studied the diffusion of tobacco mosaic virus, and Miller and Price (1946) used the method to obtain the diffusion coefficient of Southern bean mosaic virus.

Interpretation of the diffusion coefficient is fairly direct and straightforward in the cases of tomato bushy stunt virus and Southern bean mosaic virus, since these viruses are spherical The diffusion coefficient of bushy stunt virus was found to be 1.15×10^{-7} cm.^{2}/sec (Neurath and Cooper, 1940), and from this a value of 3.49×10^{-7} g/sec. can be calculated for f by means of the Einstein-Sutherland equation By making use of this value and Stokes' law the radius of bushy stunt virus was calculated to be 18.5 mμ. The diffusion coefficient of Southern bean mosaic virus was found to be 1.39×10^{-7} cm.^{2}/sec., corresponding to a radius of 15.3 mμ

SEDIMENTATION

Another method of determining the friction coefficient of a particle is through the study of its sedimentation rate However, unlike diffusion rate, *sedimentation rate does not depend solely upon f.* When a particle moves at uniform velocity through a viscous medium under the influence of a centrifugal or gravitational field, it is subjected to two equal forces, an accelerating force equal to the product of the effective mass and the acceleration of the field, and a force of resistance which is equal to the product of the velocity of the particle and f. Under such conditions one can write the equation $s = \frac{v}{g} = \frac{m_p\left(1 - \frac{d_0}{d}\right)}{f}$, in which v is the velocity of the particle, f is its friction coefficient, g is the acceleration of the field, s is the sedimentation coefficient defined as the rate of sedimentation in a field with unit acceleration, m_p is the mass of the particle, and d and d_0 are the densities of the particle and of the liquid, respectively. This equation states that the sedimentation coefficient of a particle is directly proportional to the mass of the particle corrected for the buoyancy of the liquid and inversely proportional to the coefficient of friction

It is possible to measure the sedimentation coefficient by observing the rate at which the particle moves in a field of known acceleration One merely divides the observed velocity by the acceleration to determine the sedimentation coefficient

In an ultracentrifugation experiment, the virus preparation to be studied, usually at a concentration of from 0.1 to 1 per cent, is placed in a small cell with optically perfect quartz windows in the rotor of the centrifuge. Then the rotor is spun at a known high speed, and if all the particles have exactly the same size, shape and density, all will sediment at exactly the same rate. The particles which were initially at the position nearest the axis of rotation will sediment toward the periphery at the same rate as all the other particles in the solution, but there will be no other par-

ticles to follow them. Thus, they will constitute a moving boundary between a position where there are particles and a position where there is nothing but solvent. The boundary between solvent and solution migrates toward the periphery at the rate of each particle. If the material in the preparation consists of two kinds of homogeneous particles with different sedimentation rates, two boundaries will appear as shown in Figure 5. If a large number of types of particles with slightly different sedimentation rates are present, a single boundary, which becomes blurred with time, will appear. Thus, the centrifuge can be used to determine homogeneity.

There are special optical methods which make it possible to detect a boundary between a solution and its solvent, most of which depend upon the fact that there is a refractive index gradient at such a boundary. By measuring the distance between boundary positions at two known times, it is possible to determine the velocity of migration of the boundary and hence the sedimentation coefficient.

It is relatively simple to interpret the sedimentation coefficient of a spherical particle because both the mass of the particle and the friction coefficient are directly dependent upon the radius. Hence, one can determine the radius of a spherical particle directly from its sedimentation coefficient if the density of the solvent and the density of the particle are known. The density of the dissolved material can be determined in several ways. The simplest method is to calculate it from the weight and the volume of a dry preparation. However, it is quite probable that the density of a biologic particle, such as a protein molecule or a virus particle, is not the same in solution as in the dry state owing to water of hydration.

The ultracentrifuge can be used to determine the density of a virus particle in solution. The equation $s = \dfrac{m_p\left(1 - \dfrac{d_0}{d}\right)}{f}$ shows that the sedimentation coefficient depends upon the density of the solvent. If a particle is suspended in a medium which has exactly the same density as the particle itself, it will not sediment. Therefore, if one can find a

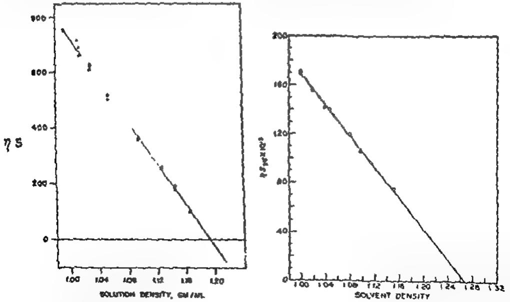


FIG. 6 The sedimentation rates of influenza (*left*) and tobacco-mosaic (*right*) viruses in sucrose solutions of different densities (Lauffer, M. A., and Taylor, N. W., Unpublished results of work on influenza A virus; Schachman, H. K., and Lauffer, M. A., 1949, The hydration size and shape of tobacco-mosaic virus, J. Am. Chem. Soc. 71, 536)

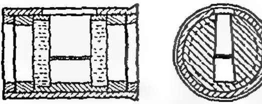


FIG 7 Longitudinal and cross-sectional views of separation cell

solvent in which the virus does not sediment, the virus will have the same density as has the solvent itself Numerous investigators have attempted to determine the density of viruses in solution by sedimenting them in solvents of different densities The method was first used by MacCallum and Oppenheimer, 1922, to estimate the density of vaccinia virus Elford and Andrewes (1936) found that influenza virus had a sedimentation coefficient of zero in a sugar solution with a density of about 1 2 The same method of approach was used by Smadel et al (1938) on vaccinia virus, by Lauffer and Stanley (1944), Sharp and associates (1944a, 1945) and Lauffer and Taylor (1953) on influenza virus, by Schachman and Lauffer (1949) on tobacco mosaic virus, and by Miller and Price (1946) and Lauffer et al. (1952) on Southern bean mosaic virus Schwerdt and Schaffer (1955) obtained a value of 1 56 for the density of poliovirus by the extrapolation of values obtained by sedimentation in mixtures of deuterium oxide-aqueous saline solutions The data obtained with tobacco mosaic virus and influenza virus are shown in Figure 6

The simplest assumption that one can make in interpreting densities so determined is that the sedimenting unit consists of virus plus water of hydration and nothing else. If the dry density of the virus is known, it is possible to calculate the hydration of the virus on the basis of this assumption The question of the interpretation of experiments such as those just described, and others, in terms of hydration, has been discussed by Lauffer and Bendet (1954).

The particles of influenza virus and Southern bean mosaic virus are essentially spherical When the value for the density of influenza virus particles in solution, 1 193 (Lauffer and Taylor, 1951), and the value of the sedimentation coefficient, 722×10^{-13} (Lauffer and Stanley, 1944), are substituted into the equation for the sedimentation of spherical particles, the diameter of the influenza particle in solution can be calculated to be 82 mμ. Values for the sedimentation coefficient and for the density in solution of Southern bean mosaic virus (Lauffer et al., 1952) are $114\ 7 \times 10^{-13}$ and 1.26, respectively. When these values are substituted into the sedimentation formula for spherical particles, a value of 28 mμ is obtained for the diameter

When one attempts to measure and to interpret the sedimentation coefficient of nonspherical particles, considerable difficulty is encountered The measurement is complicated by the fact that the sedimentation coefficient depends upon the concentration. Lauffer (1944b) showed that the reciprocal of the sedimentation coefficient of tobacco mosaic virus is linearly related to the concentration of virus In interpretation, it is necessary to obtain the sedimentation constant at infinite dilution Lauffer showed that this can be done by extrapolation of the data or by applying a correction for the viscosity of the virus solution

The interpretation of sedimentation coefficients is difficult because both f and the mass of a nonspherical particle are complex functions of its dimensions. However, a method has been evolved which makes it possible to determine the size of such particles even though the shape is not known Since both the sedimentation coefficient and the diffusion coefficient of particles are inversely proportional to f, their ratio must be independent of f and depends only upon the mass of the particle corrected for buoyancy These ideas can be summarized by the Svedberg equation: $M = \dfrac{RTs}{D(1 - d_o/d)}$ Thus, from measurements of the sedimentation coefficient, the diffusion coefficient, and the density of a particle in solution, it is possible to calculate its molecular weight regardless of its shape

The ultracentrifuge can also be used to help establish the relationship between particles in a purified preparation and the virus By means of a separation cell (Tiselius et al., 1937), Figure 7, measurements of the sedimentation rate of the entity bearing virus activity can be made The material to be studied is placed in the cell and then is spun at high speed. As

the particles bearing virus activity sediment toward the periphery, they pass through the barrier located in the center of the cell After a certain period of sedimentation, it is possible to stop the centrifuge and to withdraw the contents from the two sides of the barrier for biologic analysis From the relative amounts of biologic activity in the upper and the lower compartments, it is possible to calculate the sedimentation rate of the entity bearing virus activity This figure can be compared with the value obtained for the sedimentation coefficient of the particles If the two coincide, one has strong evidence that the virus activity is actually a property of the isolated particles, and that the particle, therefore, is the virus Evidence of this nature has been obtained for tobacco mosaic virus (Lauffer, 1943a), influenza virus (Lauffer and Miller 1944), Southern bean mosaic (Epstein and Lauffer, 1952) and poliomyelitis virus (Bachrach and Schwerdt, 1954) In the case of tobacco mosaic virus, it was found that the infectious agent sedimented at a rate which was indistinguishable from that of the particle, 15 by 300 $m\mu$ in size In a similar manner the poliomyelitis virus activity was proved to be associated with the 28 $m\mu$ particle

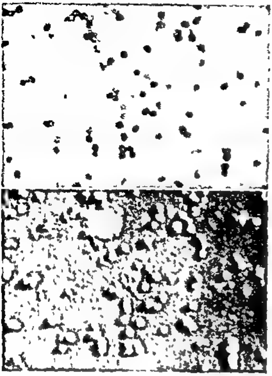
Electron Microscope

Optical Principles One of the most useful aids in the study of viruses is the electron microscope (Wyckoff, 1949, Hall, 1953) With this instrument it has been possible to obtain micrographs of many viruses both in purified form and as they exist in their intracellular environment In order to understand the principles and the operation of the electron microscope it is first necessary to examine some of the fundamentals of electron optics and also to consider the limitations of the light microscope

Although there is no theoretical upper limit to the magnification which can be secured in micrographs taken with ordinary light by the use of glass lenses, there is a distinct limit to the magnification that can be usefully employed In other words, there is a theoretical lower limit to the size of particles that can be distinguished by means of light optics When light is imaged by a lens a certain amount of diffraction occurs at its edge, resulting in phase differences in the light coming from various regions of the lens and forming the image The effect is to render impossible the imaging of a point source as a point image, instead, there is produced in the image a disk-like figure surrounded by faint interference fringes. The disk is called the "circle of confusion" If two point sources of light are very close together their circles of confusion (in the image plane) will overlap, and if the degree of overlap is great, they will be seen as a single source. To be seen as two separated points in the image it is necessary for the point sources to be far enough apart so that their circles of confusion do not overlap by more than one half

Whether or not the two circles of confusion in the image can be distinguished (i e, the two point sources can be resolved) depends upon the distance between the point sources, the wave length, λ, of light, and a property of the lens system called its numerical aperture, or N A The N A of a microscope objective lens is the product of the index of refraction of the material in the space between object and lens *times* the ratio of the lens radius to distance from lens edge to object The distance between two points that can just be resolved is given by the formula $d = \dfrac{0.6\lambda}{N\,A}$ Lenses can be constructed only with numerical apertures of 1 5 or less Therefore, the minimum resolvable distance between two object points is 0.4 λ Since ordinary visible light has a wave length of around 500 $m\mu$, the smallest resolvable distance is about 200 $m\mu$ Most viruses are smaller than this in one or more dimensions and cannot be distinguished in the ordinary light microscope

The electron microscope is in many ways analogous to the light microscope but differs in that electrons, rather than visible light, are the carriers of the radiant energy by which images are formed A source of electrons replaces the conventional light source, and magnets are used in place of glass lenses for the focusing of the electron beam to form real images The electrons from the source are usually accelerated through about 60,000 volts potential and first pass through a magnetic condenser lens that serves to control the degree of electron illumination upon the specimen Since the degree of penetration of electrons of even 60,000 volt potential is quite small, the specimen objects, such as virus particles, must be supported upon a very thin collodion membrane, which in turn is supported on a fine wire mesh When the electrons interact with the material of the specimen some of them are scattered out of the beam that finally forms the image The degree of



Fic
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owed preparation that had been treated with $CaCl_2$ to enhance contrast (*Bottom*) The same area of the preparation after being shadowed with chromium (×25,000) (Williams, R C, unpublished)

scattering depends upon the thickness of the specimen object and upon its density Contrast in the final image is thus introduced as a result of the difference in scattering power of various portions of the specimen After passing through the specimen the electron beam passes through a magnetic objective lens and a projector lens to form a real, magnified image of the object. The final image is cast upon a fluorescent screen, where it can be seen by the operator; if photographs are desired, a photographic plate replaces the screen. The interior of the entire instrument is maintained
mole-
scat-

A property of electrons that makes their use profitable for microscopy is that they possess certain wavelike characteristics, so that one may speak of the wave length of an electron beam just as one speaks of the wave length of a light beam At 60,000 volts potential the wave length is only about 0 04 Å. If the N.A. of the objective lens could be made as large as 1.0, the resolving power of the instrument would be well below atomic dimensions In actual practice the N.A must be made much smaller, and as a consequence the best resolutions obtained are in the range of 10-20 Å But even this is some 100 to 200 times better than the resolution afforded by a light microscope and is sufficiently great to permit the smallest of the known viruses to be distinguished with ease.

Limitations. There are two primary disadvantages in the examination of objects such as viruses in the electron microscope. The first arises from the necessity of maintaining a vacuum in the instrument. As a result all specimen objects must be observed in a completely dehydrated condition. A serious consequence, morphologically, of the dehydration is that objects dried directly from aqueous suspension will be seriously flattened and distorted However, this difficulty can be avoided in good part by the use of two technics designed to preserve 3-dimensional morphology. One of these is an adaption for electron microscopy (Williams, 1953) of the well-known process of lyophilization, or freeze-drying The other (Anderson, 1951) is a technic by which the specimen objects are not dried out of water but rather are "dried" from suspension in liquid CO_2 whose temperature is raised above the critical point

The second difficulty encountered in the electron microscopy of objects as small as viruses involves the consideration of image contrast When the particle is extremely small, the electron scattering due to its mass thickness may be only a fraction of that of the surrounding area of the supporting collodion film, and low contrast results A particle as small as a poliomyelitis virus, for example, will appear in a micrograph as only a dark gray object on a background almost as dark.

A method of enhancing the contrast exhibited in the images of small particles and of producing a 3-dimensional impression in the electron micrographs is called shadowing or shadow-casting (Williams and Wyckoff, 1944, 1946). In this technic a very thin film of some heavy metal, such as uranium, is evaporated in vacuo at an oblique angle upon a specimen film that carries the particles, such as viruses, upon its top surface. The evapora-

tion is done simply by heating a small bit of uranium, held in a tungsten filament, to a temperature above its boiling point. Since the atoms of the vaporized metal will travel in straight lines in a vacuum, any projecting particle upon the surface of the specimen film will intercept those atoms that otherwise would have struck the surface behind it. On the film directly behind the virus, then, there will be a region devoid of condensed uranium, while the neighboring film will receive its fair amount. A permanent, metal "shadow" will be produced, and since this region will scatter electrons less than the particle or the surroundings, it will appear black in an electron micrograph negative. Figure 8 shows an example of the increased contrast and detail made visible by shadowing.

Another method of enhancing contrast, but one that has not yet reached a high state of development, is to impregnate the specimen object with a heavy metal that will effectively increase its density (Hall, 1955). This is es- [illegible]

[illegible]

of stains employed in the former art is legion, there are scores of compounds that will react with, or impregnate, biologic materials to form a colored complex. But color, i.e., selective absorption of radiation, does not exist in the formation of electron images. There is only a limited selection of metals dense enough to affect appreciably the scattering of electrons by small objects impregnated with such metals.

Results Obtained With Electron Microscopy. The usefulness of the electron microscope in virus research has expanded rapidly and considerably during the 19 years that have elapsed since the instrument was [illegible]

[illegible]

than the structural features that are actually found on the surface of, and within, virus particles. Also, technics have improved greatly, but it still remains true that the present limitations upon the observation of virus structure are due more to the inadequacies of specimen preparation than to any lack of inherent optical power of the electron microscope. In

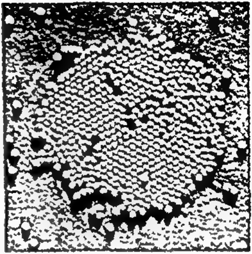

Fig. 9. A small, ordered array of particles of poliomyelitis virus. The virus is so uniform in size that it forms into 2-dimensional crystals when allowed to dry upon the specimen film. (×63,000.) (Schwerdt, C. E., Williams, R. C., Stanley, W. M., Schaffer, F. L., and McClain, M. [redacted] 1954. Morphology of type II poliomyelitis virus (MEF-1) as determined by electron microscopy. Proc. Soc. Exper. Biol. & Med. *86*, 310-312.)

recent years the greatest successes in the use of the instrument have been in the delineation of the structure of purified, intact virus particles and of virus substructures, in the exposition of virus particles in their intracellular environment, and in helping the study of the quantitative relation of virus particles to infection (see Williams, 1957, for a detailed review).

Morphology of Purified, Intact Virus Particles. Many of the viruses responsible for diseases in man, animals, higher plants, insects and bacteria have been photographed with considerable clarity in the electron microscope. Since the intact virus particles usually do not exhibit any discernible interior structure when photographed in unshadowed preparations, most work has been done on shadowed material. As can be seen in Figure 1, both the sizes and the shapes of viruses vary rather greatly. Very generally speaking, the viruses of man and animals are spherical in shape, those of higher plants are rod-shaped or polyhedral, those of insects are shaped like

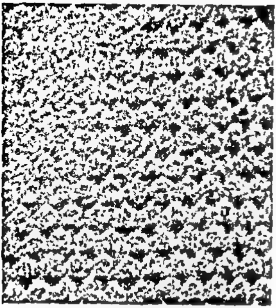

FIG 10 An electronmicrograph of a replica of a plane surface cut through the interior of a single crystal of poliomyelitis virus. (×180,000) (Steere, R L, and Schaffer, F L., 1958, The structure of crystals of purified Mahoney poliovirus, Biochim et biophys acta *28*, [No 2] 245)

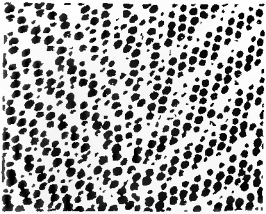

FIG 11 A thin section of a fixed, embedded crystal of the *Tipula paludosa* cytoplasmic virus showing ordered arrays (×20,000) (Williams, R. C., and Smith, K. M., 1957, A crystallizable insect virus, Nature, London *179*, 119-120)

prolate spheroids, while the bacterial viruses are sperm-shaped with polyhedral heads. While surface detail can be seen on some viruses, it is so irregular in arrangement that its significance is an enigma.

Some of the smaller viruses are so uniform in shape and size that they can be caused to aggregate into fairly large, perfect crystals When this occurs it is possible to observe their ordered structure, either by photographing a replica of their surfaces or of a plane cut through their interiors (Steere, 1957) or by photographing a thin section cut from them after fixation and embedding (Williams and Smith, 1957). Figure 9 shows a 2-dimensional array of poliomyelitis virus Figure 10 is an electron micrograph demonstrating the ordered appearance of the poliovirus particles within the interior of a large crystal such as that shown in Figure 3 Figure 11 is a micrograph of a thin section of a crystal of an insect virus

One of the most interesting findings concerning the morphology of viruses has resulted from the application of the freeze-drying and the critical-point technics. This is the demonstration that many viruses are distinctly polyhedral in shape. The heads of all frozen-dried bacterial viruses examined are so shaped, many of the plant viruses are, and even a virus as large as the cytoplasmic virus of the insect *Tipula paludosa* (Frontispiece) is exquisitely polyhedral For the last-named virus it is known that the particle is shaped like an icosahedron, a Platonic solid with 20 faces, each an equilateral triangle The importance of this discovery is its implication that a virus is synthesized from subunits that fit together in an ordered fashion to produce a particle that is in itself at least partially crystalline.

Virus Substructures. In recent years it has been possible to observe some of the smaller structures that go together to make up the intact virus particle. Such structures are obtained by either chemical or physical degradation of the native virus If some sort of chemical or biologic identification of the substructures can be secured, it is possible to obtain an enhanced understanding of the relation of viral structure and function

Work of the nature just outlined has been notably accomplished with the fowl plague virus (Schäfer and Zillig, 1954), the T_2 bacterial virus (Williams and Fraser, 1956), and with tobacco mosaic virus (TMV) (Hart, 1955). It was found that fowl plague virus could be disintegrated by exposure to ether,

following which exposure two components are obtained—a small, ribonucleoprotein particle having certain antigenic properties, and a large, protein particle possessing agglutinating properties. It was proposed that the former particle comes from the interior of the virus, while the latter comes from its outer regions. The T_4 bacterial virus was disrupted by mechanical means and was found to be primarily separable into two identifiable components—the head and the tail. The head, [illegible]

arable into a sheathlike structure, a central core, and a number of fine fibers attached to its distal end. Of particular interest is the discovery that the primary organs of attachment of the whole virus are the tail fibers (Fig. 12), the function of the core is unknown.

Considerable effort has been made to uncover the substructure of TMV. By chemical means it is possible to separate the protein from the ribonucleic acid (RNA) of the virus, and it has been found that the latter is localized co-axially along the virus rod (Fig. 13). The precise geometric fitting of the RNA into the rod is as yet unknown. The protein can be disintegrated into morphologically distinctive units, each of which is a disklike section with a hole in the center, from which the RNA has presumably escaped. The intact virus rod itself has an axial hole, but it is of smaller diameter than the one seen in the disintegrated protein portion. An important finding is that the RNA alone is somewhat infectious, although the protein is not (Fraenkel-Conrat, 1956, Gierer and Schramm, 1956, Fraenkel-Conrat et al., 1957). It is possible to reconstitute the intact virus rod, with almost full return of infectivity, by mixing under proper conditions the previously separated RNA and protein fractions (Fraenkel-Conrat and Wil-

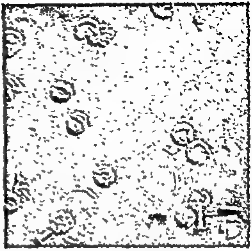

FIG. 12. An electronmicrograph showing some of the substructures of the T2 bacteriophage. Four "ghosts," with tails attached to head membranes, are seen, as well as several head membranes devoid of tails. Also shown are V-shaped fibers that serve as the primary seat of phage attachment. (×48,000.) (Williams, R. C., and Fraser, D., 1956. Structural and functional differentiation in T2 bacteriophage. Virology 2, 289-307.)

FIG. 13. A display of TMV substructures. (*Left*) an intact TMV [illegible]

liams, 1955, Fraenkel-Conrat, 1957). The protein alone can be polymerized into rods (Schramm, 1943). Lauffer et al. (1958) discovered that polymerization involves absorption of heat and must, therefore, be accompanied by an increase in entropy.

Intracellular Viruses. Although information of great value to virologists has been obtained through electron microscopy of purified, intact viruses, and of their substructures, it is evident that a subject of more directly biologic interest is the investigation of viruses in their intracellular environment. Special techniques have been developed to extend the classic methods of histologic preparation to meet the special requirements of electron microscopy. Two requirements of paramount importance are that (1) the fixatives and the stains employed must yield finely granular backgrounds, and (2) the embedding and sectioning methods must produce sections that are extremely thin, on the order of 20 to 50 mμ in thickness. It is found that buffered osmium tetroxide is a satisfactory fixative, with some staining propensities for lipids and proteins. Although its action is not well understood, its practical

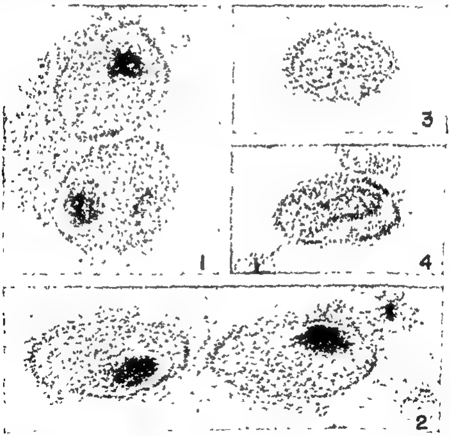

FIG. 14. Vaccinia and fowlpox viruses in thin sections. (1) Two vaccinia

velopmental stage. An elongated internal body is surrounded by dense material. These particles are eccentric to the plane of section and therefore appear small. (4) Fowlpox virus at a similar stage. In contrast with vaccinia virus the internal body is separated from the peripheral membrane by a zone of low density. (×130,000) (From Morgan, C., and Rose, H. M., unpublished)

usefulness is unchallenged. Embedding is accomplished in one of the thermo setting plastics, usually butyl methacrylate, while the sectioning is done with specially designed microtomes provided with knives of broken glass, or, recently, of polished diamond

In accord with expectation, the viruses that have proved to be the most amenable to observation are the largest ones or the ones that grow abundantly in the diseased cells and tissues. The viruses well examined in sections are those of the psittacosis-lymphogranuloma group, the pox group, the viruses of influenza, herpes simplex, and the adenovirus group. Without exception these have exhibited a somewhat elaborate but characteristic structure when seen in section within infected cells (Morgan et al, 1954a, 1954b, 1955, 1956) There is a central, electron-dense core, surrounded by one or two concentric membranes Figure 14 and 15 show the appearance of vaccinia virus, fowlpox virus and an adenovirus in intracellular form. The last virus is particularly interesting in that, when within the nucleus, it forms into large well-ordered arrays Influenza gives evidence of proliferating in a somewhat unexpected manner. It is rarely, if ever, found well within the cytoplasm, but rather it appears to be formed as a particle only as it is being extruded from the cell surface

The purpose of looking for viruses in sections of cells is to determine their sites of proliferation and to arrive at some notion as

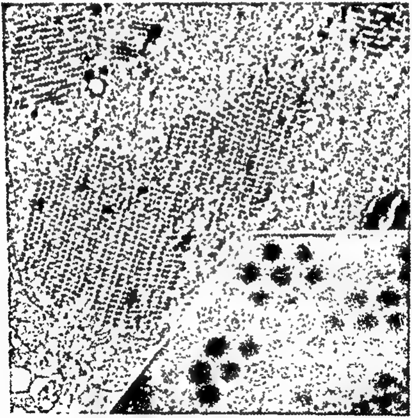

Fig. 15. A thin section [illegible]
Th[illegible]
In[illegible]
vir[illegible] with a center to center spacing of c. 650 A. Several particles exhibit internal structure (×140,000) (From Morgan, C., and Rose, H. M., unpublished)

to their changes in structure as they are synthesized within the cell It is clear that the former purpose can be satisfied only if there is unequivocal knowledge as to which of the numerous particles seen in sections of cells are viral in nature With the largest viruses, this problem of identification is trivial, but as the smaller viruses are sought, it becomes a difficult, and as yet unsolved, one In order to arrive at conclusions about the changes of virus appearance as the disease progresses within the cells, it is necessary, or at least desirable, to have some measure of time sequence within the infected material With gross tissue involvement there can be no certainty that all cells are infected at the same time, and the questions as to what represents an "early" and what a "late" infection becomes subjective guesswork Recent advances in the use of cultured cells, particularly of single cells, should help to reduce both of the uncertainties discussed.

Quantitative Applications. The electron microscope may be put to considerable use as an instrument for counting the number of virus particles present in a suspension (Backus and Williams, 1950). The method employed is quite simple. A virus preparation is mixed with a suspension of indicator particles, such as spheres of polystyrene latex, whose absolute number concentration is known The mixture is then sprayed upon the specimen films for subsequent electron microscopy. The small spray-drops upon drying form minute drop patterns (Fig 16) that can, and must, be photographed in their entirety. A simple

Fig. 16 Droplet pattern of poliomyelitis virus mixed with a suspension of polystyrene latex indicator particles at a concentration of 3.20×10^{10} particles/ml. The large, opaque spheres are the polystyrene latex particles ($\times$13,500) (From Schwerdt, C. E, unpublished)

counting of the numbers of virus and of indicator particles allows the ratio of their numbers to be determined. This ratio, multiplied by the number concentration of the indicator particles, yields directly the number concentration of the virus particles in suspension. Since this method provides a determination of the number of physical particles present, and biologic titrations can provide a measure of the number of infectious units present in another sample of the same suspension, it is possible to secure an important virologic parameter: the number of physical virus particles necessary for one infectious dose or the specific infectivity. With some bacterial viruses and with vaccinia this number is close to unity (Luria et al., 1951; Overman and Tamm, 1956), with influenza it is probably about 10 (Donald and Isaacs, 1954), while with poliomyelitis it is about 40 (Schwerdt and Fogh, 1957). (See Isaacs, 1957, for a critical review.)

The application of the counting method provides a criterion of viral identification. If an infective preparation, when sprayed and micrographed, is seen to be monodisperse, it is likely that the particles seen are those of the virus. However, if the drop-patterns exhibit two or more distinctive particle types, the question arises as to which particle type is to be associated with the virus. But for each type an apparent specific infectivity can be calculated following biologic titration. The procedure then is to treat the preparation by chemical or physical means such as to change the relative concentrations of the types of particles present. New apparent specific infectivities are obtained, and that particle type for which this parameter remains constant is the most likely candidate for consideration as the virus. This is the method that was employed in the first identification of poliomyelitis virus (Bachrach and Schwerdt, 1954).

X-Ray Diffraction

When a beam of monochromatic light coming from a narrow slit is passed through a diffraction grating and then is focused upon a screen, the image of the light source is found to consist of a series of lines. The central line will be the brightest and will be the principal image of the slit source. Above and below it will be other images of lower intensity. The distance between the successive images depends upon the distance between the diffraction grating and the screen, upon the distance between the lines on the diffraction grating, and upon the wave length of the light. If one knows the wave length of the light and the distance between the grating and the screen, one can calculate the distance between the lines on the grating.

FIG. 17. Longitudinal and cross-sectional representations of arrangement of rod-shaped tobacco-mosaic virus in rodlike crystals. (Lauffer, M. A., and Stanley, W. M., 1939, The physical chemistry of tobacco-mosaic virus protein, *Chem. Rev. 24*, 303-321.)

In a somewhat analogous way, when a narrow x-ray beam is passed through a crystal, the beam is diffracted. The crystal can be considered as a 3-dimensional diffraction grating, because the individual molecules are arranged in a regular 3-dimensional network. The type of diffraction pattern produced with such a 3-dimensional diffraction grating is far more complex than that produced by a 2-dimensional grating consisting of ruled lines on a glass plate. A great number of diffraction spots will be obtained on the photographic plate in the 3-dimensional case. However, the distance between the principal image of the x-ray source and these spots is related to the distance between molecules in the crystal in much the same way as the distance between the lines on a simple diffraction grating is related to the grating spacing. Thus, from an x-ray diffraction pattern of a crystal, one can measure the distance between molecules in that crystal. If it is assumed that the molecules are tightly packed in the crystal, then one can assume that the diameter of the molecule is equal to the shortest distance between molecules. Hence, from x-ray diffraction patterns obtained on crystals one can calculate molecular sizes. The x-ray method has been used by Bernal and Fankuchen (1941) to assess the size of the tomato bushy stunt virus and also to determine the diameter of the tobacco mosaic virus rods. Figure 17 represents diagrammatically the arrangement of rod-shaped tobacco mosaic virus particles in the rodlike crystals of the virus. The particles are arranged with perfect hexagonal sym-

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of unchanged particles remaining. A result of this sort is conventionally ascribed to a one-hit process.

In spite of the fact that this inactivation is a one-hit phenomenon, the efficiency of inactivation was found to be extremely small. The result was obtained by comparing the total amount of ultraviolet light absorbed by the virus protein at wave length 2537 A with the number of particles inactivated. It was found that something like 25,000 photons are absorbed on the average by each particle which becomes inactivated. Yet, it has been seen that only one of these photons is effective in inactivating the particle.

The action of ionizing radiations on biologic materials, including viruses, has been reviewed by Lea (1947) and by Pollard (1953). The energy from ionizing radiations is absorbed by the irradiated material by the mechanism of ionization. When a tissue is irradiated with x rays or with gamma rays, the photons of radiation collide with atoms and cause ionization by knocking out electrons with very high energies. These electrons or elementary particles of negative electricity speed through the tissue and cause secondary ionizations along their path. Secondary ionization results when an electron is moved out of the orbit of one atom, leaving a positive ion behind, and is captured by another atom, producing a negative ion. The production of each ion pair results in the dissipation of some of the energy of the primary electron, and ultimately all of the energy is dissipated, and the primary electron stops. These ion pairs are produced along tracks, i.e., along the path of the primary ionizing particle. The density of ion production is inversely related to the energy of the primary ionizing electron. Beta rays from radioactive decay or cathode rays are high-speed electrons. These behave in exactly the same manner as the ionizing electrons which result from absorption of x rays or gamma rays. Alpha particles, which are helium nuclei and are produced as a result of radioactive decay, directly induce ion pairs in the tissue. However, alpha particles are very efficient ionizers and therefore produce very dense ionization along a relatively short path. High-speed protons, which can be obtained from a cyclotron, behave somewhat like alpha particles but produce ionization densities intermediate between those of alpha particles and electrons. Neutrons, which can be obtained from the cyclotron, collide with an atom and knock out a proton. This proton in turn behaves in the same way as a proton coming originally from the cyclotron.

It takes energy to produce an ion pair. Ultimately, the excess electrons on the negative members of the ion pairs will find their way back to the electron-deficient orbits of the positive members of the pair, and, when this happens, energy is made available at that spot. This energy leaves the molecule or chemical grouping which contained the ionized atom in a high-energy or excited state, capable of undergoing reaction. If the chemical reaction is in a configuration essential for biologic function, the particle loses its biologic activity.

Irradiation studies on biologic materials, including viruses, are sometimes carried out with the material in solution. In such a case the effect of the radiation can be either direct or indirect, i.e., an ion pair can be produced in the biologic particle itself and directly cause an effect, or it can be produced in the solvent leading to the formation of some substance in the solvent, probably a free radical, which is detrimental to the biologically active material. This indirect effect of radiation can, in principle, be suppressed by irradiating only highly concentrated suspensions or by adding substances like gelatin to the solution for the purpose of absorbing the bulk of the detrimental substances produced. The reality of this distinction between indirect and direct effects as applied to viruses has been demonstrated by x-ray inactivation studies carried out on T_2 bacteriophage by Buzzell and Lauffer (1952) and on tobacco mosaic virus by Buzzell et al. (1956).

When a sample of virus is irradiated in the dry state or when adequate precautions are exercised during the irradiation of solutions, it is usually assumed that the effects observed are the result of direct ionizations within the virus particle. It is customary to interpret the direct action of radiations in terms of the so-called target theory. When a virus is irradiated with a particle which produces dense ion tracks, i.e., tracks along which the ion pairs are so dense that the distance between them is small compared with the size of a virus particle, then the probability that an ionization will be produced within a particular virus particle depends only upon the cross-sectional area of the virus particle and upon

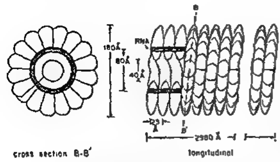

Fig 18 Schematic representation of a tentative structure for tobacco mosaic virus (Stanley, W M, 1956, Virus composition and structure—25 years ago and now, Fed Proc. *15*, 812-818)

metry with respect to cross-section, but with no regularity with respect to length.

The evidence from x-ray analysis as well as that from electron microscopy indicates that the center-to-center separation of the rods of tobacco mosaic virus is 150 Å when the rods are packed in either a 2-dimensional or 3-dimensional array A recent study of the fine structure of the x-ray patterns of the crystalline virus indicates strongly that the exterior of the rods is deeply grooved (Franklin and Klug, 1956). This could account for the observation from radial distribution functions that the total diameter of the rods is as great at 180 Å but that upon close-packing screw-wise intermeshing might occur and thus permit a center-to-center distance of only 150 Å. The localization of the ribonucleic acid in the virus rod has been determined by comparing the radial distribution of electron density of the virus rod with that of the polymerized protein fraction containing no nucleic acid. The difference in the two distributions presumably locates the x-ray scattering electrons in the virus nucleic acid. These results indicate that the nucleic acid, presumably the phosphate groups, is localized some 40 Å from the axis of the rod at a distance about halfway between the axis and the mean outer surfaces (Caspar and Franklin, 1956). There is also evidence from this work that the virus rod has a hole along its axis of radius about 20 Å A schematic representation of a tentative structure for the tobacco mosaic virus molecule is shown in Figure 18. The protein subunits of molecular weight about 17,000 are arranged in the form of a helix with a pitch of 23 Å and with 49 subunits in a length of 69 Å along the rod (Franklin, 1956).

Irradiation

It is well known that viruses can be inactivated or changed in other ways as a result of the effects of various kinds of radiations To understand these effects, it is necessary to differentiate between those radiations which excite atoms and those which ionize atoms Visible and ultraviolet light produce their effect by excitation; alpha, beta, gamma and x-rays, accelerated neutrons, protons and deuterons are ionizing radiations

The effect of nonionizing or exciting radiations on viruses and other biologic materials has been reviewed by McLaren (1949). The effects produced by radiations of this sort represent a special aspect of photochemistry. Experience with photochemical reactions extending over more than a century has led to the formulation of two laws: (1) only light absorbed can cause a reaction; (2) in the primary process one quantum of light is absorbed by each molecule which reacts A molecule which has absorbed a quantum of radiation has a higher energy level than its neighbors. In this high-energy state, it and the group of molecules of which it is a part are likely to undergo chemical change. If the chemical configuration which changes is one of those essential to some vital function, then a biologic change results.

Ultraviolet-irradiation studies have been carried out on many viruses and bacteriophages, e g , equine encephalomyelitis, herpes, influenza, poliomyelitis, vaccinia, rabies, tobacco mosaic and tomato bushy stunt viruses, and *Staphylococcus aureus* and *E. coli* bacteriophages When tobacco mosaic virus was irradiated with ultraviolet light of wave length 2537 Å, it was found that the logarithm of the relative infectivity remaining was inversely proportional to the radiation dose (Oster and McLaren, 1950). This is the kind of result that one gets whenever the rate of the change being measured is directly proportional to the number of individuals remaining unchanged If the absorption of a single photon of radiation is sufficient to cause a virus particle to become inactivated, then the probability of a particular virus particle being hit in a short time interval will be a constant when the radiation intensity is maintained constant The number of virus particles changing in that time will depend only upon the number

of unchanged particles remaining. A result of this sort is conventionally ascribed to a one-hit process.

In spite of the fact that this inactivation is a one-hit phenomenon, the efficiency of inactivation was found to be extremely small. The result was obtained by comparing the total amount of ultraviolet light absorbed by the virus protein at wave length 2537 Å with the number of particles inactivated. It was found that something like 25 000 photons are absorbed on the average by each particle which becomes inactivated. Yet, it has been seen that only one of these photons is effective in inactivating the particle.

The action of ionizing radiations on biologic materials, including viruses, has been reviewed by Lea (1947) and by Pollard (1953). The energy from ionizing radiations is absorbed by the irradiated material by the mechanism of ionization. When a tissue is irradiated with x-rays or with gamma rays, the photons of radiation collide with atoms and cause ionization by knocking out electrons with very high energies. These electrons or elementary particles of negative electricity speed through the tissue and cause secondary ionizations along their path. Secondary ionization results when an electron is moved out of the orbit of one atom, leaving a positive ion behind, and is captured by another atom, producing a negative ion. The production of each ion pair results in the dissipation of some of the energy of the primary electron, and ultimately all of the energy is dissipated and the primary electron stops. These ion pairs are produced along tracks, i.e., along the path of the primary ionizing particle. The density of ion production is inversely related to the energy of the primary ionizing electron. Beta rays from [illegible]

[illegible]

or gamma rays. Alpha particles, which are helium nuclei and are produced as a result of radioactive decay, directly induce ion pairs in the tissue. However, alpha particles are very efficient ionizers and therefore produce very dense ionization along a relatively short path. High-speed protons, which can be obtained from a cyclotron, behave somewhat like alpha particles but produce ionization densities intermediate between those of alpha particles and electrons. Neutrons, which can be obtained from the cyclotron, collide with an atom and knock out a proton. This proton in turn behaves in the same way as a proton coming originally from the cyclotron.

It takes energy to produce an ion pair. Ultimately, the excess electrons on the negative members of the ion pairs will find their way back to the electron-deficient orbits of the positive members of the pair, and, when this happens, energy is made available at that spot. This energy leaves the molecule or chemical grouping which contained the ionized atom in a high-energy or excited state, capable of undergoing reaction. If the chemical reaction is in a configuration essential for biologic function, the particle loses its biologic activity.

Irradiation studies on biologic materials, including viruses, are sometimes carried out with the material in solution. In such a case the effect of the radiation can be either direct or indirect, i.e., an ion pair can be produced in the biologic particle itself and directly cause an effect, or it can be produced in the solvent leading to the formation of some substance in the solvent, probably a free radical, which is detrimental to the biologically active material. This indirect effect of radiation can, in principle, be suppressed by irradiating only highly concentrated suspensions or by adding substances like gelatin to the solution for the purpose of absorbing the bulk of the detrimental substances produced. The reality of this distinction between indirect and direct effects as applied to viruses has been demonstrated by x-ray inactivation studies carried out on T_2 bacteriophage by Buzzell and Lauffer (1952) and on tobacco mosaic virus by Buzzell et al. (1956).

When a sample of virus is irradiated in the dry state or when adequate precautions are exercised during the irradiation of solutions, it is usually assumed that the effects observed are the result of direct ionizations within the virus particle. It is customary to interpret the direct action of radiations in terms of the so-called target theory. When a virus is irradiated with a particle which produces dense ion tracks, i.e., tracks along which the ion pairs are so dense that the distance between them is small compared with the size of a virus particle, then the probability that an ionization will be produced within a particular virus particle depends only upon the cross-sectional area of the virus particle and upon

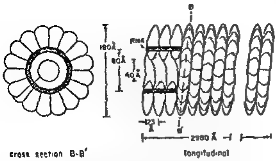

Fig 18. Schematic representation of a tentative structure for tobacco mosaic virus (Stanley, W M , 1956, Virus composition and structure—25 years ago and now, Fed Proc *15*, 812-818)

metry with respect to cross-section, but with no regularity with respect to length.

The evidence from x-ray analysis as well as that from electron microscopy indicates that the center-to-center separation of the rods of tobacco mosaic virus is 150 Å when the rods are packed in either a 2-dimensional or 3-dimensional array A recent study of the fine structure of the x-ray patterns of the crystalline virus indicates strongly that the exterior of the rods is deeply grooved (Franklin and Klug, 1956) This could account for the observation from radial distribution functions that the total diameter of the rods is as great at 180 Å but that upon close-packing screw-wise intermeshing might occur and thus permit a center-to-center distance of only 150 Å. The localization of the ribonucleic acid in the virus rod has been determined by comparing the radial distribution of electron density of the virus rod with that of the polymerized protein fraction containing no nucleic acid. The difference in the two distributions presumably locates the x-ray scattering electrons in the virus nucleic acid. These results indicate that the nucleic acid, presumably the phosphate groups, is localized some 40 Å from the axis of the rod at a distance about halfway between the axis and

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Irradiation

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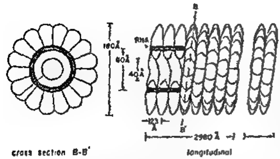

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IRRADIATION

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by the method of light-scattering (Oster, 1946; Oster et al., 1947) The method and its biophysical and biochemical applications have been reviewed by Edsall and Dandliker (1951). Particles too small to resolve light might nevertheless scatter it The extent of scattering (therefore, the turbidity) depends upon several factors, among which are the concentration and the molecular weight of the dissolved particles, the refractive indices of solvent and solution and the wave length of light. Thus, from turbidity measurements and measurements of the concentration of the material it is possible to determine the molecular weight For the study of particles much smaller than the wave length of light, the experimental arrangements can be very simple Most of the scattered light will not be transmitted through the solution in its original direction Hence, the ratio of the intensity of light transmitted by the solvent to that of light transmitted by suspension of virus particles is related directly to the turbidity. Transmitted light can be measured in an ordinary laboratory colorimeter Thus, the method of light-scattering may involve only apparatus which is commonly available in clinical laboratories, and it does give satisfactory results for the size of spherical viruses With an apparatus which permits observation of scattering at different angles it is possible to investigate particles having at least one dimension as great as or greater than the wave length of light Oster et al (1947) determined both the molecular weight and the lengths of tobacco virus particles and obtained values in agreement with those determined by other methods The theory of light-scattering parallels that of osmotic pressure For this reason it is possible to investigate the interaction between virus particles or other particles in solution by measuring light-scattering at various concentrations.

Electrophoresis

Viruses, because of their chemical composition, possess carboxyl, amino and other groups capable of ionizing as acids or as bases The dissociation of carboxyl and other acidic groups is suppressed by the addition of strong acids to the medium The addition of strong bases causes the suppression of the ionization of the amino and other basic groups Thus, it can be seen that the relative degree of dissociation of the acidic and the basic groups on the surface of a virus particle and hence its electric charge depends upon the pH of the medium When the protein contains an equal number of dissociated acidic and basic groups, it will have a net electric charge of zero and will be in the iso-electric state

An electric current is carried through a solution of ions by the migration of the ions. Charged macromolecules also migrate in an electric field and thereby help to transport current The velocity of migration of the macromolecules per unit of field strength, generally called the electrophoretic mobility, depends upon the net charge which is a function of pH, upon the electrolyte concentration and the viscosity of the medium, and upon the size and the shape of the charged particles Since the surface chemical composition and the size and the shape of various proteins and viruses differ widely, it is only natural that individual proteins and viruses should have distinctive mobilities in the same solvent Thus, electrophoretic mobility can be used as a means of identifying a protein or a virus and also as a means of separating it from other proteins

Electrophoresis experiments are traditionally carried out in the Tiselius apparatus (Tiselius, 1937, Longsworth 1942), the essential component of which consists of a U-shaped channel of rectangular cross-section connected at each end to a nonpolarizable electrode After a sharp boundary between virus solution and buffer solution is established in each arm of the U-tube, the electrodes are connected to a source of electric potential, and current is sent through the U-tube. If the [illegible] the [illegible] ele[illegible] identical, all will migrate at the same rate Thus, the sharp boundary between solution and solvent will be maintained The U-tube is placed in a thermostatically controlled water bath equipped with a specialized optical system which enables the boundary to be seen and its motion analyzed as with boundaries in the ultracentrifuge

Electrophoresis has been used to purify viruses Price (1946) observed that some preparations of Southern bean mosaic virus contained a dark-brown pigment which could not be removed readily. It was found by Lauffer and Price (1947) that in a 0.02 M

TABLE 2 CORRESPONDENCE BETWEEN X-RAY TARGET AND NUCLEIC ACID VOLUME OF VIRUSES

VIRUS	TARGET VOLUME / TOTAL VOLUME	NUCLEIC ACID VOLUME / TOTAL VOLUME
Tobacco necrosis	0 18	0 18
Tobacco ring spot	0 35	0 40
Tomato bushy stunt	0 18	0 17
E coli bacteriophage T_2	0 34	0 37-0 50
T_6	0 34	0 37
T_7	0 37	0 38
T_5	0 39	—
Shope papilloma	0 081	0 087
PR8 influenza	0 065	0 05

(Buzzell, A , Trkula, D , and Lauffer, M A , 1956, X-ray studies on tobacco mosaic virus, Arch Biochem & Biophys , *63*, 470-476)

the number of ion tracks per unit of cross-sectional area in the irradiated material This situation would obtain when viruses are irradiated with alpha particles. The fraction of the number of viable virus particles which become inactivated in a given small unit of time then would depend solely upon the dose rate of irradiation and upon the effective cross-sectional area of the virus particle When the dose rate is known, then it is possible in principle to calculate the cross-sectional area from the observed rate of inactivation

When a virus is irradiated with a sparsely ionizing radiation, such as high energy x-rays or gamma rays, then the probability of inactivation depends on the probability of an ionization occurring within the target In this case, fractional biologic response depends on the dose and on the target volume

If inactivation is the result of a single event, i e , either the production of a single ionization within the volume of a single virus particle or the intersection of a particle with one ion track, then the dose-response curve should be a straight line when the logarithm of response is plotted against the dose Many viruses have been irradiated with various kinds of ionizing radiations Representative studies are described by Lea (1947) and by Pollard (1951). In general, the dose-response curves obtained are of the single-hit type Also in the earlier studies, it seemed that the effective particle sizes calculated from such studies are about the same as the sizes of the characteristic particles of the virus as determined by means of electron microscopy and other physical methods

This problem was studied by Epstein (1953), who proposed that the correspondence between nucleic acid volume and target volume for many viruses was so much better than the correspondence between target volume and total volume that it seemed proper to conclude that the real target for the action of x-rays was the nucleic acid Buzzell et al (1956) recalculated some of the data used by Epstein in the light of more refined measurements and of their modification of the theory It can be seen from the results shown in Table 2 that the correspondence between target volume and nucleic acid volume is remarkably good

Lauffer et al. (1956) isolated the nucleic acid from samples of tobacco mosaic virus which had been irradiated with doses up to 8 times the average lethal dose and showed that this material had a much lower intrinsic viscosity than the nucleic acid isolated from unirradiated tobacco mosaic virus This experiment indicates that x-rays may produce their lethal effect by breaking a nucleic acid chain Ginoza and Norman (1957) have shown that the x-ray inactivation dose for isolated TMV nucleic acid is the same as that for the intact TMV.

LIGHT-SCATTERING METHODS

The sizes of the characteristic particles of influenza virus, tomato bushy stunt virus and tobacco mosaic virus have been determined

appear first in the effluent liquid, and others will follow in sequence

An alternative way of operating a column is to cause a large volume of a solution, perhaps containing several substances, to flow through the column and then analyze the effluent liquid on the other end The various constituents of the solution will then be retarded by the amount required to saturate the column The extent of retardation of the appearance of a component depends upon the degree to which it is adsorbed and upon its concentration Thus, if one analyzes the effluent liquid, one will find that it is free of a particular component for a time, and then suddenly this component appears Chromatography experiments carried out in this way are called "frontal analysis" experiments (Tiselius, 1947)

When filter paper is used as the adsorbent, the usual technic is to place a small streak of a solution containing several components near the bottom of a strip of filter paper The pure solvent is allowed to rise by capillary action through the strip Then the components in the streak are separated into bands along the paper, the position of the band depending upon how strongly a particular substance is adsorbed At the end of the experiment, the bands can be located by the use of dyes, by ultraviolet light or, if the substances happen to be colored, by ordinary visual observation The paper can be cut into pieces, and in this way the components of any one band can be recovered for analysis

Paper chromatography has been applied to the study of viruses Gray (1952) studied tobacco mosaic virus, and Goldstein (1956) Southern bean mosaic virus by this technic

Numerous studies have been carried out using ion exchange resins for the purification of viruses Matheka and Armbruster (1956) and other workers used a strongly basic resin for the purification of influenza virus, and Shainoff and Lauffer (1956) used such a resin for the purification of Southern bean mosaic virus Bradley and Rich (1955) and Commoner et al (1956) used an anion exchange cellulose preparation for the separation of nucleic acid and protein degradation products of tobacco mosaic virus Tiselius (1954) carried out chromatography of tobacco mosaic virus on calcium phosphate

Shainoff and Lauffer (1957) used frontal analysis chromatography on a strongly basic anion exchange resin for the purpose of identifying the infectious entity with the characteristic particle of Southern bean mosaic virus The retention of the infectious entity was the same as that of the nucleoprotein under all of the conditions studied Because of the sharpness of the boundaries of the fronts, the experimental error in the retention volume of the infectious entity was not greatly influenced by the accuracy of the bio-assay. Thus, evidence for the identity of the infectious entity with the characteristic particle was obtained

Chemistry

Chemical Analysis Elementary chemical analyses have been made on purified preparations of several viruses and several strains of the same virus Unfortunately, such data are not very informative, since about all that can be concluded is that the data are compatible with what might be expected for the general class of nucleoproteins The plant viruses appear to be the least complex chemically, for all which have been purified have been found to be simple nucleoproteins The amount of nucleic acid has been found to vary from about 6 per cent in the case of tobacco mosaic virus to about 35 per cent in the case of tobacco ring spot and turnip yellow mosaic viruses (Bawden and Pirie, 1937, Stanley, 1939, Stanley and Loring, 1939, Ross and Stanley, 1939, Steere, 1956, Cosentino et al, 1956) The Shope rabbit papilloma virus appears to contain about 1 5 per cent of lipid in addition to nucleoprotein (Taylor et al, 1942) Influenza virus has been found to contain, in addition to nucleoprotein, a polysaccharide composed of mannose, galactose, fucose and glucosamine units, and lipid (Ada and Gottschalk, 1956, Knight, 1947a, Frommhagen et al, 1958) Equine encephalomyelitis virus was found to contain large amounts of lipid in the form of phospholipid, cholesterol and neutral fat (Beard, 1945) The viruses which have been purified and studied have been found to consist of various combinations of nucleic acid and protein with lipid, extranucleic acid carbohydrate or certain other components which are present in some instances Thus far, only pentose nucleic acid has been found in the plant viruses, while either pentose nucleic acid or deoxypentose

TABLE 3

Virus	Isoelectric Point pH	Reference
Tobacco mosaic	3 49	Eriksson-Quensel and Svedberg, 1936
PR8 influenza A	5 30	Miller, Lauffer and Stanley, 1944
Tomato bushy stunt	4 11	McFarlane and Kekwick, 1938
Rabbit papilloma	5 0	Sharp et al , 1942
Southern bean mosaic	5 9	MacDonald, Price and Lauffer, 1949

phosphate buffer at pH 7, this pigment migrated more rapidly in electrophoresis than did the Southern bean mosaic virus, thus allowing a separation to be made Schramm et al (1955) used electrophoresis to purify the protein fraction isolated from tobacco mosaic virus

The electrophoresis apparatus can also be used for determining the electrophoretic homogeneity of a virus preparation by observing whether or not the boundaries are single or multiple, or whether they are sharp or diffuse, as in the analysis of boundaries in the ultracentrifuge Electrophoresis experiments may also be carried out on filter paper A virus solution is placed on a strip of filter paper saturated with buffer, and the strip is allowed to make contact with electrodes at the two ends. When an electric current is passed through the strip, the protein material migrates electrophoretically at a characteristic rate Gray (1952) carried out such studies on tobacco mosaic virus, cucumber mosaic virus and tomato spotted wilt virus. Zaitlin (1956) used filter paper electrophoresis to separate strains of tobacco mosaic virus

One of the characteristic physical constants by which a virus can be identified is its isoelectric point. At the iso-electric point, it will not migrate either to the positive or the negative electrode in an electrophoresis apparatus. The iso-electric point can be determined by measuring the electrophoretic mobility in buffers with different pH values and establishing the pH at which there is no migration To- phoretically homogeneous. Their isoelectric points have the values indicated in Table 3 The isoelectric point may vary with the concentration of the buffer, as was shown by Hartman and Lauffer (1953) for Southern bean mosaic virus, Different strains of the same virus may have different iso-electric points, as was shown for tobacco mosaic virus by Oster (1951), by Gordon and Price (1953), and by Ginoza and Atkinson (1955)

Electrophoresis can also be used to help establish the identity between a particle and the infectious entity in a manner analogous to the procedure used with the centrifuge. Hartman and Lauffer (1953) found that over a large pH range the electrophoretic mobility of the infectious entity in a Southern bean mosaic virus preparation coincided with that of the characteristic particle Miller et al. (1944) found that the red blood cell precipitating activity of influenza virus migrated in an electric field with the same rate as the particles commonly regarded as the influenza virus particle

CHROMATOGRAPHY

It has been mentioned earlier that viruses can be adsorbed to and eluted from various finely divided solid materials. When the adsorption and elution process is carried out by the method of moving the material through a long column packed with the adsorbent or along a strip of filter paper, it is given the name, "chromatography"

There are several ways of carrying out chromatographic operations One is to place on a tightly packed column of adsorbent a small amount of a solution containing several components Then solvent is forced through the column, and this causes the original band of solutes to move in the direction of flow The less strongly adsorbed substances move more rapidly than the more strongly adsorbed After a time, the components of the solution are separated into distinct bands distributed along the column. If the components happen to be colored, these bands can be seen directly Frequently, a band will be found to contain a single pure substance One way of recovering pure material is to divide the column into sections corresponding to the various bands Another way is to allow solvent to continue flowing; the most rapidly moving band will come to the end of the column and

strains of tobacco mosaic virus (Reddi, 1957). The polynucleotide chains of tobacco mosaic virus nucleic acid seem to be terminated by 3'-phosphomonoester groups (Markham et al., 1954; Reddi and Knight, 1957). Because the methods that were employed formerly to secure the nucleic acid components of viruses for chemical studies were not designed to retain biologic activity, it is probable that some of these preparations were altered considerably. Cohen and Stanley (1942) found that the nucleic acid obtained by alkali treatment of tobacco mosaic virus was only slightly viscous and possessed a molecular weight of about 15,000, whereas the nucleic acid obtained by heat treatment was very viscous, spontaneously birefringent and possessed a molecular weight of about 300,000. Fraenkel-Conrat and Singer (1958) have studied the effects of different solvents, metals and chelating agents on the biologic activity of nucleic acid preparations obtained by treatment of tobacco mosaic virus with either sodium dodecyl sulfate or phenol.

Fraenkel-Conrat and Williams (1955) found that tobacco mosaic virus nucleic acid would enter into combination with the viral protein subunits to form rods 15 $m\mu$ [redacted] diameter, many with lengths and specific viral activity similar to those of the intact virus. The fact that the virus activity per unit weight of nucleic acid is increased from 20-fold to 100-fold when the nucleic acid is incorporated in the protein, and that the nucleoprotein has a stability much greater than that of the free nucleic acid indicates that the protein is of importance in the infectious process. Hart and Smith (1956) have found that different artificial and natural ribonucleotide polymers would interact with tobacco mosaic virus protein to form nucleoprotein rods somewhat similar to those of tobacco mosaic virus but possessing no virus activity. Fraenkel-Conrat and Singer (1957) have studied the reconstitution of virus particles from protein and nucleic acid preparations of different virus strains and have found that the nature of the disease and the progeny produced by such mixed viruses appeared to be governed by the nucleic acid component. Since mixtures of nucleic acids may be packaged together, this may provide a new approach to the study of genetic recombination in viruses. The discovery that tobacco mosaic virus nucleic acid possesses virus activity by itself and that the virus nucleoprotein can be reconstituted from protein and nucleic acid subunits has opened up many new research opportunities of great potential in virus research.

Chemical Modification. In early studies on the inactivation of tobacco mosaic virus, [redacted] was found that treatment with hydrogen peroxide, formaldehyde or nitrous acid yielded inactive virus which still retained certain characteristic chemical and serologic properties (Stanley, 1936). The reaction with formaldehyde was studied in some detail by Ross and Stanley (1938), who found that the inactiva-
[illegible]

formaldehyde was dialyzed [redacted] pH 3, a 10-fold greater activity was obtained following dialysis than before. This was interpreted to mean that the virus was reactivated by dialysis. This difference was also found to be accompanied by differences in amino nitrogen and in the groups that react with Folin's reagent. Kassanis and Kleczkowski (1944) reported that they were unable to secure reactivation of formolized virus, but the experiments of Ross and Stanley have been repeated, and the results confirmed in another laboratory (Fischer and Lauffer, 1949a). Heicken and Spicher (1956) found that formaldehyde inactivated T_3 bacteriophage preparations could be largely reactivated by treatment with amino acids such as asparagin, tryptophan or histidine.

Considerable progress has been made toward understanding the way in which tobacco mosaic virus is inactivated by formaldehyde as a result of studies of the kinetics of the reaction. Ross and Stanley (1938) showed that the inactivation of tobacco mosaic virus by formaldehyde is a reaction of the first order over most of the course of inactivation, and this was confirmed by Fischer and Lauffer (1949b). A first-order reaction is one in which the amount of primary reactant remaining unchanged decreases exponentially with time. This type of reaction implies that the number of particles changing in a very short interval of time is directly proportional to the number remaining unchanged. When one plots

nucleic acid or both have been reported to be present in animal viruses.

Although all strains of tobacco mosaic virus were found to contain the same amount of nucleic acid, as indicated by phosphorus analyses, they possessed different chemical properties (Stanley, 1937a, 1943). The properties of different preparations of tobacco mosaic virus obtained at different times of the year and even from different kinds of hosts were found to be identical by all tests applied (Stanley and Loring, 1936, Stanley, 1938b, Gaw and Stanley, 1947) The first studies on the amino acid composition of different strains of tobacco mosaic virus were made by isolation and colorimetric methods but later were supplemented by microbiologic and chromatographic methods (Ross and Stanley, 1939; Ross, 1941b, 1942, Knight, 1942, 1947b; Black and Knight, 1953, Ramachandran, 1957) Differences in the composition of the 8 strains were found to involve 16 of the 19 amino acids that were determined In some cases, a strain is characterized by changes in the amount of one or more amino acids, by the introduction of a new amino acid or by the elimination of an amino acid On the other hand, deFremery and Knight (1955) in a study of purified preparations of 3 strains of tomato bushy stunt virus found no decisive differences in amino acid composition Despite the profound differences in composition that have been found in strains of tobacco mosaic virus, the particles of these strains appear to have the same size and shape.

At the present time attention is being turned toward the determination of amino acid sequences in the proteins of tobacco mosaic virus strains Preliminary results indicate that differences in sequence occur in different strains of this virus (Niu and Fraenkel-Conrat, 1955, Knight, 1956, Narita, 1957). Niu et al (1958) have found the C-terminal residues of tomato bushy stunt virus, potato X virus and the cucumber viruses 3 and 4 to be leucine, proline and alanine, respectively. No free amino terminal groups were found

Amino acid analyses have also been made on highly purified preparations of the PR8 strain of influenza A virus, the Lee strain of influenza B virus (Knight, 1947b), and the Shope rabbit papilloma virus (Knight, 1950) Although purified tobacco mosaic virus and its strains were found to be unrelated immunochemically to the proteins of the normal hosts used to produce the viruses, the influenza viruses were found to be related immunochemically to materials present in the hosts (Chester, 1935, Knight, 1946a, b, Gaw and Stanley, 1947, Malkiel, 1947).

The significance of chemical differences in the protein components of viruses was altered somewhat with the very important discovery by Fraenkel-Conrat (1956), and independently and almost simultaneously by Gierer and Schramm (1956), that nucleic acid preparations possessing virus activity could be obtained by degradation of the tobacco mosaic virus nucleoprotein It now appears that the nucleic acid alone carries the biochemical message which results in the production in an appropriate host not only of a specific nucleic acid but also of a specific protein which eventually serves as a protective coat for the biologically active nucleic acid. This conclusion is consistent with earlier work which indicated that deoxyribonucleic acids carried the transforming activity of the pneumococcus (Avery et al, 1944) and was also responsible for phage replication activity (Hershey and Chase, 1952) It is obvious that great emphasis must now be placed on studies of viral nucleic acids

The nucleic acid component of tobacco mosaic virus has been studied by Loring (1939) and by Schwerdt and Loring (1947), and it was concluded that at least 3 of the component nucleotides were identical with those of yeast nucleic acid Knight (1952) studied the molar proportions of the bases in strains of tobacco mosaic virus and found no differences. However, the immunologically related cucumber 3 and 4 viruses were found to contain decidedly less adenine and more uracil Differences in base ratios were also found in the cases of tomato bushy stunt, Southern bean mosaic and potato X viruses (Markham and Smith, 1951; Dorner and Knight, 1953). Ribonuclease treatment has yielded no evidence for differences in the composition of the ribonuclease-resistant cores of nucleic acids from strains of tobacco mosaic virus (Reddi and Knight, 1956), although evidence has been obtained that the intramolecular distribution of pyrimidine nucleotides in the masked strain differs from those of other

viruses when treated in a similar manner with carboxypeptidase.

The use of stable and radioactive isotopes in research work on viruses will doubtless yield important results. Stanley (1942b) and Schramm et al (1942) prepared tobacco mosaic virus containing radioactive phosphorus Although the phosphorus did not dissociate from the virus in vitro, it was found to be dissociable following inoculation into plant cells; hence, the virus containing radioactive phosphorus did not prove to be useful in connection with experiments designed to yield information concerning the mode of virus reproduction However, it does appear likely that the use of tracer technics may provide an answer to the important question as to whether or not the substance of virus used as inoculum appears in the progeny or newly formed virus particles Important advances have already been made in this direction in studies on the reproduction of bacteriophages using radiophosphorus, radiosulfur, radiocarbon and heavy nitrogen Hershey et al (1951) found that phage preparations containing a high specific activity of incorporated radiophosphorus are inactivated progressively by the decay of their radioactive atoms, and Stent (1955) subsequently observed that at the outset of their intracellular reproduction such P^{32}-labeled phages become refractory to inactivation by radioactive decay One of the most significant isotope experiments was carried out by Hershey and Chase (1952) who demonstrated that it is only the viral phosphorus (i e , DNA) and not the viral sulfur (i e , protein) which penetrates into the interior of the infected bacterium Putnam and Kozloff (1950) discovered that about half of the DNA so introduced into the host cell by the parent virus is ultimately transferred to the progeny virus particles In this process of transfer from parent to progeny, the phosphorus atoms of the parental DNA become distributed over several progeny particles, as could be shown by means of direct autoradiographic measurements (Levinthal, 1956) or by the lethal effects produced by the transferred P^{32} atoms (Stent and Jerne, 1955)

Dunn and Smith (1954) and Litman and Pardee (1956) have studied the incorporation of 5-bromouracil into bacteriophages The latter workers found that 5-bromouracil has a powerful mutagenic effect, presumably because of a disturbance of deoxyribonucleic acid metabolism. The percentage of mutants is about 15, the highest as yet recorded.

Kinetics of Disintegration

It has been found that tobacco mosaic virus is disintegrated very much more rapidly at extremely high pressures than at ordinary pressures (Lauffer and Dow, 1941). That is, even though the virus is perfectly stable at 30° C. at atmospheric pressure, it will disintegrate if it is subjected to a pressure of from 5,000 to 10,000 atmospheres at 30° C. The course of the disintegration at high pressures has been shown to be of the first order. The effect of high pressures on the disintegration of tobacco mosaic virus can be understood on the basis of the assumption that the partially denatured virus particles occupy slightly less volume than the normal virus particles If this is so, then one would expect the application of high pressures to shift the equilibrium in such a way as to increase the proportion of particles in the partially denatured state Thus, the reaction rate would be speeded up More recently, Johnson et al (1948) showed that at pressures around 500 atmospheres the denaturation of tobacco mosaic virus protein was less rapid than at atmospheric pressure This result indicates that the denaturation processes at 500 and at 7,500 atmospheres are not the same

The disintegration of tobacco mosaic virus by urea presents some very interesting features (Stanley and Lauffer, 1939, Lauffer, 1943b, Lauffer and Stanley, 1943) When the virus is placed in a strong urea solution, it gradually disintegrates This reaction has been shown to be of the first order The specific reaction rate was found to vary with the temperature in an unexpected and unusual manner The disintegration of tobacco mosaic virus in urea proceeds slowly at room temperature, but the rate is increased when the temperature is raised above room temperature and also when it is lowered below room temperature. Other viruses have been shown to behave in the same manner (Bawden and Pirie, 1940) This very unexpected behavior can be explained on the basis of the assumption that the virus reacts with urea to form a complex compound which then undergoes disintegration By making the postulate that at least two different kinds of complexes can

the logarithm of the amount remaining against time, the slope of the resulting straight line is called "the specific reaction rate"

It is commonly observed that, when the natural logarithm of the specific reaction rate for many chemical reactions is plotted against the reciprocal of the absolute temperature, the data fit a straight line From the slope of this straight line, one can evaluate the energy of activation In studies on the inactivation of tobacco mosaic virus by formaldehyde at several pH values Cartwright et al. (1956) found energies of activation of approximately 19,500 cal/mole. This value is approximately that which one obtains with ordinary chemical reactions involving a single chemical bond and *would indicate that the rate-determining step* involves a single chemical bond. The same conclusion is indicated by the fact that the rate of inactivation is directly proportional to the first power of formaldehyde concentration *This is not to say that only one formal*dehyde molecule reacts with a virus particle Indeed, there are undoubtedly thousands of formaldehyde molecules combined with each virus particle after the reaction is completed. It means only that, of the large number of formaldehyde molecules which do react, a single one is responsible for the loss of biologic activity Fraenkel-Conrat (1954) found that formaldehyde can react with the nucleic acid of the intact tobacco mosaic virus, and Staehelin (1957) in studies with the separated nucleic acid found experimentally that only about 3 formaldehyde molecules need to be bound to render the nucleic acid inactive It is not unreasonable to conclude that re*action of a single formaldehyde molecule* at a single site on the nucleic acid of the virus may cause inactivation

The studies of Cartwright et al (1956) show that the rate of the inactivation is independent of pH over the range pH 4-8. This can be interpreted to mean that the critical group for inactivation in the presence of formaldehyde is one which does not ionize in this pH range Possibilities are the OH group on the ribose and the NH_2 of the purine and pyrimidine residues

Anson and Stanley (1941) reported that the sulfhydryl groups of tobacco mosaic virus could be oxidized with iodine without changing the specific virus activity, but that the inoculation of this oxidized virus in Turkish tobacco plants resulted in the production of ordinary tobacco mosaic virus Fraenkel-Conrat (1955) found that iodine transforms the sulfhydryl of tobacco mosaic virus into a stable sulfenyl iodide group which becomes unstable upon denaturation of the virus Carpenter and associates (1948) treated tobacco mosaic virus with benzyl or n-butyl β-chloroethyl sulfide and found that the vesicant residues were attached to both the nucleic acid and the protein moieties of the virus and that the vesicant-protein linkage was more labile to alkali than was the vesicant-nucleic acid linkage. Miller and Stanley (1941, 1942) found that chemical derivatives of tobacco mosaic virus could be obtained by treatment with ketene, phenyl isocyanate, carbobenzoxy chloride, p-chlorobenzoyl chloride and benzenesulfonyl chloride Knight (1951) prepared dinitrophenyl derivatives of tobacco mosaic virus and Fraenkel-Conrat (1953) was able *to introduce an amino acid, namely, leucine,* into the virus structure. It is of interest that the coverage of functional groups which can be accomplished without change in specific virus activity, as measured on leaves of *Nicotiana glutinosa,* corresponds to about 3,000 amino groups and from 2,000 to 4,000 phenolic groups per molecule of tobacco mosaic virus In the light of the recent work with infectious nucleic acid it is not surprising that the disease caused by these chemical derivatives in Turkish tobacco plants was found to be indistinguishable from the ordinary tobacco mosaic disease, and that the virus isolated from such plants was found to be indistinguishable from ordinary tobacco mosaic virus.

Harris and Knight (1955) studied the action of carboxypeptidase on tobacco mosaic virus and found that about 2,900 threonine residues and only theronine were released per *molecule of virus Further, it was found* that the biologic properties and the size, the shape and the density of the treated virus were the same as those of the original virus. Knight (1955) also found that the same amount of threonine and only threonine was split from each of 13 strains of tobacco mosaic virus on treatment with carboxypeptidase In contrast, smaller quantities of several amino acids were split from potato X, Southern bean mosaic, tomato bushy stunt and tobacco ring spot

Brakke, M. K , Vatter, A [redacted], and Black, L M., 1954, Size and shape of wound-tumor virus, [redacted] Abnormal and Pathological Plant Growth, Brookhaven Symposia in Biology No 6, pp 137-156

Buzzell, A., and Lauffer, M A , 1952, X-ray studies on T_3 bacteriophage, Arch Biochem *39*, 195-204

Buzzell, A., Trkula, D, and Lauffer, M A , 1956, X-ray studies on tobacco mosaic virus, Arch Biochem *63*, 470-476

Carpenter, F H., Wood, J L., Stevens, C M, and du Vigneaud V., 1948 Chemical studies on vesicant-treated proteins, J Am Chem Soc *70*, 2551

Cartwright, T E, Ritchie, A E, and Lauffer, M A , 1956, The reaction of tobacco mosaic virus with formaldehyde III Kinetics of the loss of infectivity Virology *2*, 689-702

Caspar, [redacted] L D , and Franklin, [redacted] E , 1956, Structure of tobacco mosaic virus 1 Radial density distribution in the tobacco mosaic virus particle 2 Location of the ribonucleic acid in the tobacco mosaic virus particle Nature, London *177*, 928-930

Chester, K S , 1935 The antigenicity of the plant viruses, Phytopathology *25*, 702-714

Cohen S S , and Stanley, W M , 1942, The molecular size and shape of the nucleic acid of tobacco mosaic virus, J Biol Chem *144*, 589-598

Commoner, B , Lippincott, J A , Shearer, G [redacted] Richman, [redacted] E , and Wu, J -H 1956, Reconstitution of tobacco mosaic virus components Nature, London *178*, 767-771

Cosentino, V , Paigen, K , and Steere, R L , 1956, Electron microscopy of turnip yellow mosaic virus and the associated abnormal protein, Virology *2*, 139-148

Cox, [redacted] R., van der Scheer J , Aiston, S , and Bohnel, E., 1947, The purification and concentration of influenza virus by means of alcohol precipitation, J Immunol *56*, 149-166

Cunha, R , Weil, M L , Beard, D , Taylor, A R , Sharp, [redacted] G , and Beard, J W 1947, Purification and characters of the Newcastle disease virus (Calif strain), J Immunol *55*, 69-89

deFremery, D , and Knight, C A , 1955, A chemical comparison of three strains of tomato bushy stunt virus, J Biol Chem *214*, 559-566

Donald, [redacted] B , and Isaacs, A , 1954, Counts of influenza particles, J Gen Microbiol *10*, 457-464

Dorner, R W , and Knight, C A , 1953, The preparation and properties of some plant virus nucleic acids, J Biol Chem *205*, 959-967

Duffy, C E , and Stanley, W M , 1945, Studies on the biochemical, biophysical, and immunogenic properties of Japanese B type encephalitis virus and vaccines, J Exper Med *82*, 385-410

Dunn, [redacted] B , and Smith, J D , 1954, Incorporation of halogenated pyrimidines into the deoxyribonucleic acids of *Bacterium coli* and its bacteriophages, Nature, London *174*, 305

Edsall, J T , and Dandliker, W B , 1951, Light scattering in solutions of proteins and other large molecules Its relation to molecular size and shape and molecular interactions Fortschr d Chem Forsch *2*, 1-56

Elford, W J , 1933, Principles of ultrafiltration as applied in biological studies, Proc Roy Soc , London, B, *112*, 384-406

Elford, W J , and Andrewes, C H , 1932, Filtration of vaccinia virus through gradocol membranes, Brit J Exper Path *13*, 36-42

———, 1936, Centrifugation studies II The viruses of vaccinia, influenza, and Rous sarcoma Brit J Exper Path *17*, 422-430

Elford, W J , Andrewes, C H , and Tang, F. F., 1936, The sizes of the viruses of human and swine influenza as determined by ultrafiltration, Brit J Exper Path *17*, 51-53

Epstein, H T , 1953, Identification of radiosensitive volume with nucleic acid volume, Nature, London *171*, 394

Epstein, H T , and Lauffer, M A., 1952, Ultracentrifugal identification of infectious entities with characteristic particles, Arch Biochem *36*, 371-382

Eriksson-Quensel, I B., and Svedberg, T , 1936, Sedimentation and electrophoresis of the tobacco mosaic virus protein, J Am Chem Soc *58*, 1863-1867

Fischer, M A , and Lauffer, M A., 1949a, The reaction of tobacco mosaic virus with formaldehyde I Electrophoretic studies Arch Biochem *23*, 291-296

———, 1949b, The reaction of tobacco mosaic virus with formaldehyde II Kinetics J Am Chem Soc *71*, 3800-3804

Fraenkel-Conrat, H., 1953, The reaction of proteins with ^{14}C-labelled N-carboxyleucine anhydride, Biochim et biophys acta *10*, 180-182

———, 1954, Reaction of nucleic acid with formaldehyde, Biochim et biophys acta *15*, 308-309

———, 1955, The reaction of tobacco mosaic virus with iodine, J Biol Chem *217*, 373-381

———, 1956, The role of the nucleic acid in the reconstitution of active tobacco mosaic virus, J Am Chem Soc *78*, 882

———, 1957, The infectivity of tobacco mosaic virus nucleic acid Cellular biology, nucleic acids and viruses, Special Publications of the New York Acad Sc *5*, 217-225

Fraenkel-Conrat, H , and Singer, B , 1957, Virus reconstitution II Combination of protein and nucleic acid from different strains Biochim et biophys acta *24*, 540-548

———, 1958, Effect of salt concentration on the infectious nucleic acid from tobacco mosaic virus In preparation

Fraenkel-Conrat, H , Singer, [redacted] , and Williams, [redacted] C , 1957, Infectivity of viral nucleic acid, Biochim et biophys. acta *25*, 87-96

Fraenkel-Conrat, H., and Williams, R C , 1955, Reconstitution of tobacco mosaic virus from its inactive protein and nucleic acid components, Proc Nat Acad Sc *41*, 690-698

Franklin, [redacted] E , 1956, X-ray diffraction studies of cucumber virus 4 and three strains of tobacco mosaic virus, Biochim et biophys acta *19*, 203-211

Franklin, R E , and Klug, A , 1956, The nature of the helical groove on the tobacco mosaic virus particle, Biochim et biophys acta *19*, 403-416

be formed with urea, one can explain the unusual dependence of reaction rate upon temperature

Rather extensive studies on the kinetics of the destruction of infectivity of tobacco mosaic virus and of other plant viruses were carried out by Price (1940) In general, it was found that these inactivations follow the course of a first-order reaction The kinetics of the loss of red blood cell agglutinating activity and of infectivity of the PR8 strain of influenza A virus has been studied by Lauffer and associates (Lauffer and Carnelly, 1945, Lauffer and Wheatley, 1951, Lauffer et al, 1948, Scott and Lauffer, 1946a, b). Studies of this sort are of importance to virologists because they shed light upon the inactivation of viruses at high temperatures During much of the early work, a given virus was characterized by its inactivation temperature or its thermal death point This was defined as the temperature at which a virus would be inactivated in some arbitrary time It is now realized that the thermal inactivation of a virus is merely one aspect of the broader problem of the denaturation or disintegration of virus proteins Inactivation is a reaction which takes place over a wide range of temperatures. However, the reaction has the property of changing velocity greatly for small temperature changes Thus, a change of temperature of only a few degrees can cause the reaction velocity to change from an imperceptibly low value to one so high that virtually all of the activity is destroyed in a short period of time Hence, one can obtain an apparent inactivation temperature. These inactivation temperatures or thermal death points were used as characteristics to describe the nature of a particular virus Much more useful information can be obtained from data describing the way in which the rate of the inactivation varies with temperature and with other conditions.

REFERENCES

(For complete references, see also 2nd edition, Chapter 2)

Ada, G L, and Gottschalk, A, 1956, The component sugars of the influenza-virus particle, Biochem J *62*, 686-689

Anderson, T F, 1946, Morphological and chemical relations in viruses and bacteriophages, Cold Spring Harbor Symp on Quant Biol *11*, 1-13

———, 1951, Techniques for the preservation of three-dimensional structure in preparing specimens for electron microscopy, Tr. New York Acad Sc *13*, 130-134

Anson, M L, and Stanley, W M, 1941, Some effects of iodine and other reagents on the structure and activity of tobacco mosaic virus, J Gen Physiol *24*, 679-690

Avery, O T, MacLeod, C M, and McCarty, M, 1944, Studies on the chemical nature of the substance inducing transformation of pneumococcal types Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III J Exper. Med *79*, 137-158

Bachrach, H L, and Schwerdt, C E, 1954, Purification studies on Lansing poliomyelitis virus II Analytical electron microscopic identification of the infectious particle in preparations of high specific infectivity J Immunol *72*, 30-38

Backus, R. C, and Williams, R C, 1950, The use of spraying methods and of volatile suspending media in the preparations of specimens for electron microscopy, J Appl Physics *21*, 11-15

Bang, F. B, 1946, Filamentous forms of Newcastle virus, Proc Soc Exper. Biol & Med *63*, 5-7

Bawden, F C, and Pirie, N W, 1937, The isolation and some properties of liquid crystalline substances from solanaceous plants infected with three strains of tobacco mosaic virus, Proc Roy Soc, London, s B, *123*, 274-320

———, 1940, The inactivation of some plant viruses by urea, Biochem J *34*, 1258-1277

Beard, J W, 1945, The ultracentrifugal, chemical and electron micrographic characters of purified animal viruses, Proc Inst Med Chicago *15*, 294-313

———, 1956, Virus of avian myeloblastic leukosis, Poultry Sc *35*, 203-223

———, 1957, Isolation and identification of tumor viruses, Texas Rep Biol & Med *15*, 627-658

Beard, J W, Bryan, W R, and Wyckoff, R W G, 1939, The isolation of the rabbit papilloma virus protein, J Infect Dis *65*, 45-52

Bergold, G, 1947, Die Isolierung des Polyeder-Virus und die Natur der Polyeder, Ztschr f Naturforsch *2b*, 122-143

Bernal, J D, and Fankuchen, I, 1941, X-ray and crystallographic studies of plant virus preparations I Introduction and preparation of specimens II Modes of aggregation of the virus particles III J. Gen Physiol *25*, 111-165

Black, F L, and Knight, C A, 1953, A comparison of some mutants of tobacco mosaic virus, J Biol Chem *202*, 51-57

Bradley, D. F, and Rich, A, 1955, Heterogeneity in ribonucleic acids. Abstracts of Papers, 128th Meeting, American Chemical Society, Minneapolis, Minn, Sept 11-16, 1955, p 3C

Brakke, M K, Black, L M, and Wyckoff, R W G, 1951, The sedimentation rate of potato yellow-dwarf virus, Am J. Bot *38*, 332-342

Brakke, M K, Vatter, A E, and Black, L M, 1954, Size and shape of wound tumor virus, *in Abnormal and Pathological Plant Growth*, Brookhaven Symposia in Biology No 6, pp 137-156

Buzzell, A, and Lauffer, M A, 1952, X-ray studies on T_3 bacteriophage, Arch Biochem *39*, 195-204

Buzzell, A, Trkula, D, and Lauffer, M A, 1956, X-ray studies on tobacco mosaic virus, Arch Biochem *63*, 470-476

Carpenter, F. H, Wood, J L, Stevens, C M, and du Vigneaud V, 1948, Chemical studies on vesicant-treated proteins, J Am Chem Soc *70*, 2551

Cartwright, T F, Ritchie, A E, and Lauffer, M A 1956, The reaction of tobacco mosaic virus with formaldehyde III Kinetics of the loss of infectivity, Virology *2*, 689-702

Caspar, D L, D, and Franklin, R E 1956, Structure of tobacco mosaic virus 1 Radial density distribution in the tobacco mosaic virus particle 2 Location of the ribonucleic acid in the tobacco mosaic virus particle Nature, London *177*, 928-930

Chester, K S, 1935, The antigenicity of the plant viruses, Phytopathology *24*, 702-714

Cohen, S S, and Stanley, W M, 1942, The molecular size and shape of the nucleic acid of tobacco mosaic virus, J Biol Chem *144*, 589-598

Commoner, B, Lippincott, J A, Shearer, G B Richman, E E, and Wu, J-H, 1956, Reconstitution of tobacco mosaic virus components, Nature, London *178*, 767-771

Cosentino, V, Paigen, K, and Steere, R L, 1956, Electron microscopy of turnip yellow mosaic virus and the associated abnormal protein, Virology *2*, 139-148

Cox, H, R, van der Scheer, J, Aiston, S, and Bohnel, E, 1947, The purification and concentration of influenza virus by means of alcohol precipitation, J Immunol *56*, 149-166

Cunha, R, Weil, M L, Beard, D, Taylor, A R, Sharp, D G, and Beard, J W 1947 Purification and characters of the Newcastle disease virus (Calif. strain), J Immunol *55*, 69-89

deFremery, D, and Knight, C A, 1955, A chemical comparison of three strains of tomato bushy stunt virus, J Biol Chem *214*, 559-566

Donald, H B, and Isaacs, A, 1954, Counts of influenza particles, J Gen Microbiol *10*, 457-464

Dorner, R W, and Knight, C A, 1953, The preparation and properties of some plant virus nucleic acids, J Biol Chem *205*, 959-967

Duffy, C E, and Stanley, W M, 1945, Studies on the biochemical, biophysical, and immunogenic properties of Japanese B type encephalitis virus and vaccines, J Exper Med *82*, 385-410

Dunn, D B, and Smith, J D, 1954, Incorporation of halogenated pyrimidines into the deoxyribonucleic acids of *Bacterium coli* and its bacteriophages, Nature, London *174*, 305

Edsall, J T, and Dandliker, W B, 1951, Light scattering in solutions of proteins and other large molecules Its relation to molecular size and shape and molecular interactions Fortschr d Chem Forsch *2*, 1-56

Elford, W J, 1933 Principles of ultrafiltration as applied in biological studies, Proc Roy Soc, London, B, *112*, 384-406

Elford W J, and Andrewes, C H, 1932, Filtration of vaccinia virus through gradocol membranes, Brit J Exper Path *13*, 36-42

———, 1936, Centrifugation studies II The viruses of vaccinia influenza, and Rous sarcoma Brit J Exper Path *17*, 422 430

Elford, W J, Andrewes, C H, and Tang, F F, 1936, The sizes of the viruses of human and swine influenza as determined by ultrafiltration, Brit J Exper Path *17*, 51-53

Epstein, H T, 1953, Identification of radiosensitive volume with nucleic acid volume, Nature, London *171*, 394

Epstein, H T, and Lauffer, M A, 1952, Ultracentrifugal identification of infectious entities with characteristic particles, Arch Biochem *36*, 371-382

Eriksson-Quensel, I B, and Svedberg, T, 1936, Sedimentation and electrophoresis of the tobacco mosaic virus protein, J Am Chem Soc *58*, 1863-1867

Fischer, M A, and Lauffer, M A, 1949a, The reaction of tobacco mosaic virus with formaldehyde I Electrophoretic studies Arch Biochem *23*, 291-296

———, 1949b The reaction of tobacco mosaic virus with formaldehyde II Kinetics J Am Chem Soc *71*, 3800-3804

Fraenkel-Conrat, H, 1953, The reaction of proteins with ^{14}C-labelled N-carboxyleucine anhydride, Biochim et biophys acta *10*, 180-182

———, 1954, Reaction of nucleic acid with formaldehyde, Biochim et biophys acta *15*, 308-309

———, 1955, The reaction of tobacco mosaic virus with iodine, J Biol Chem *217*, 373-381

———, 1956, The role of the nucleic acid in the reconstitution of active tobacco mosaic virus, J Am Chem Soc *78*, 882

———, 1957, The infectivity of tobacco mosaic virus nucleic acid Cellular biology nucleic acids and viruses, Special Publications of the New York Acad Sc *5*, 217-225

Fraenkel-Conrat, H, and Singer, B, 1957, Virus reconstitution II Combination of protein and nucleic acid from different strains Biochim et biophys acta *24*, 540-548

———, 1958, Effect of salt concentration on the infectious nucleic acid from tobacco mosaic virus In preparation

Fraenkel-Conrat, H, Singer, B, and Williams, R C, 1957, Infectivity of viral nucleic acid, Biochim et biophys acta *25*, 87-96

Fraenkel-Conrat, H, and Williams, R C, 1955, Reconstitution of tobacco mosaic virus from its inactive protein and nucleic acid components, Proc Nat Acad Sc *41*, 690-698.

Franklin, R E, 1956, X-ray diffraction studies of cucumber virus 4 and three strains of tobacco mosaic virus, Biochim et biophys acta *19*, 203-211

Franklin, R E, and Klug, A, 1956, The nature of the helical groove on the tobacco mosaic virus particle, Biochim. et biophys acta *19*, 403-416.

Frommhagen, L H, Freeman, N K, and Knight, C A, 1958, The lipid constituents of influenza virus, chick allantoic membrane, and sedimentable allantoic protein Virology, in press

Gaw, H Z, and Stanley, W M, 1947, Comparative properties of purified preparations of two distinctive strains of tobacco mosaic virus obtained from diseased Turkish tobacco and phlox plants, J Biol Chem *167, 765-772*

Gierer, A, and Schramm, G, 1956, Die Infektiosität der Nucleinsäure aus Tobakmosaikvirus, Ztschr f Naturforsch *11b, 138-141*

Ginoza, W, and Atkinson, D E, 1955, Comparison of some physical and chemical properties of eight strains of tobacco mosaic virus, Virology *1, 253-260*

Ginoza, W, and Norman, A, 1957, Radiosensitive molecular weight of tobacco mosaic virus nucleic acid, Nature, London *179, 520*

Goldstein, I F, 1956, Paper Chromatography of Plant Viruses and Lipoproteins M S Thesis, University of Pittsburgh

Gordon, R B, and Price, W C, 1953, Differentiation of tobacco mosaic virus strains by differences in pH with maximum optical densities, Arch Biochem *45, 117-123*

Gray, R A, 1952, The electrophoresis and chromatography of plant viruses on filter paper, Arch Biochem *38, 305-316*

Green, R H, Anderson, T F, and Smadel, J E, 1942, Morphological structure of the virus of vaccinia, J Exper Med *75, 651-656*

Hall, C E, 1953, Introduction to Electron Microscopy, New York, McGraw-Hill

———, 1955, Electron densitometry of stained virus particles, J Biophys & Biochem Cytol *1, 1-12*

Harris, J I, and Knight, C A, 1955, Studies on the action of carboxypeptidase on tobacco mosaic virus, J Biol Chem *214, 215-230*

Hart, R G, 1955, Electron-microscopic evidence for the localization of ribonucleic acid in the particles of tobacco mosaic virus, Proc Nat Acad Sc *41, 261-264*

Hart, R G, and Smith, J D, 1956, Interactions of ribonucleotide polymers with tobacco mosaic virus protein to form virus-like particles, Nature, London *178, 739-740*

Hartman, R E, and Lauffer, M A, 1953, An application of electrophoresis to the identification of biological activity with characteristic particles, J Am Chem Soc *75, 6205-6209*

Hershey, A D, and Chase, M, 1952, Independent functions of viral protein and nucleic acid in growth of bacteriophage, J Gen Physiol *36, 39-56*

Hershey, A D, Kamen, M D, Kennedy, J W, and Gest, H, 1951, The mortality of bacteriophage containing assimilated radioactive phosphorus, J Gen Physiol *34, 305-319*

Hirst, G K, 1941, The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus, Science *94, 22-23*

Hook, A. E., Beard, D, Taylor, A R, Sharp, D G, and Beard, J W, 1946, Isolation and characterization of the T_2 bacteriophage of *Escherichia coli*, *J Biol Chem 165, 241-258.*

Isaacs, A, 1957, Particle counts and infectivity titrations for animal viruses, *in* Advances in Virus Research, vol 4, pp 111-158, New York, Acad Press

Johnson, F H, Baylor, M B, and Fraser, D, 1948, The thermal denaturation of tobacco mosaic virus in relation to hydrostatic pressure, Arch Biochem *19, 237-245*

Kahn, D S, and Polson, A, 1947, A new method for forming sharp boundaries in diffusion experiments, J. Phys & Colloid Chem. *51, 816-825*

Kassanis, B, and Kleczkowski, A, 1944, The effect of formaldehyde and mercuric chloride on tobacco mosaic virus, Biochem J *38, 20-24*

Knight, C. A, 1942, Basic amino acids in strains of tobacco mosaic virus, J. Am Chem Soc *64, 2734-2736*

———, 1946a, Precipitin reactions of highly purified influenza viruses and related materials, J. Exper Med *83, 281-294*

———, 1946b, The preparation of highly purified PR8 influenza virus from infected mouse lungs, J Exper Med. *83, 11-24*

———, 1947a, The nucleic acid and carbohydrate of influenza virus, J Exper Med *85, 99-116*

———, 1947b, Amino acid composition of highly purified viral particles of influenza A and B, J Exper Med *86, 125-129*

———, 1950, Amino acids of the Shope papilloma virus, Proc Soc Exper Biol & Med *75, 843-846*

———, 1951, Dinitrophenyl derivatives of some strains of tobacco mosaic virus, J Biol Chem *192, 727-731*

———, 1952, The nucleic acids of some strains of tobacco mosaic virus, J Biol Chem *197, 241-249*

———, 1955, The action of carboxypeptidase on strains of tobacco mosaic virus, J Biol Chem *214, 231-237*

Lamm, O, 1937, Measurements of concentration gradients in sedimentation and diffusion by refraction methods Solubility properties of potato starch Nova Acta Regiae Soc. Sci Upsaliensis *[4]*, No 6, p 10.

Lauffer, M A, 1943a, The sedimentation rate of the infectious principle of tobacco mosaic virus, J Biol Chem *151, 627-634.*

———, 1943b, Denaturation of tobacco mosaic virus by urea. II Kinetic aspects, J Am Chem Soc *65, 1793-1802*

———, 1944a, The size and shape of tobacco mosaic virus particles, J. Am Chem Soc *66, 1188-1194*

———, 1944b, The influence of concentration upon the sedimentation rate of tobacco mosaic virus, J. Am Chem Soc *66, 1195-1201*

Lauffer, M A, Ansevin, A T, Cartwright, T E, and Brinton, C C, Jr, 1958, The polymerization-depolymerization of tobacco mosaic virus protein, *Nature 181*, 1338-1339

Lauffer, M. A, and Bendet, I J, 1954, The hydration of viruses, *in* Advances in Virus Research, vol 2, pp 241-287, New York, Acad Press

Lauffer, M A, and Carnelly, H L, 1945, Thermal destruction of influenza A virus hemagglutinin I The kinetic process Arch Biochem *8, 265-274.*

Lauffer, M A, Carnelly, H L., and MacDonald, E, 1948, Thermal destruction of influenza A virus infectivity, Arch Biochem *16*, 321-328

Lauffer, M A, and Dow, R B, 1941, The denaturation of tobacco mosaic virus at high pressures, J Biol Chem *140*, 509-518

Lauffer, M A., and Miller, G L, 1944, The sedimentation rate of the biological activities of influenza A virus, J Exper Med *80*, 521-529

Lauffer, M A., and Price, W C, 1947, Electrophoretic purification of Southern bean mosaic virus, Arch Biochem *15*, 115-124

Lauffer, M A., and Stanley, W M, 1943, The denaturation of tobacco mosaic virus by urea I Biochemical aspects Arch Biochem *2*, 413-424

———, 1944, Biophysical properties of preparations of PR8 influenza virus, J Exper Med *80*, 531-548

Lauffer, M A., and Taylor, N W, 1953, The influence of the composition of the medium upon the sedimentation rate of proteins II Studies on influenza virus characteristic particles Arch Biochem *42*, 102-105.

Lauffer, M A, Taylor, N W., and Wunder, C C, 1952, The influence of the composition of the medium upon the sedimentation rate of proteins I Studies on Southern bean mosaic virus protein Arch Biochem *40*, 453-471

Lauffer, M A, Trkula, D, and Buzzell, A, 1956, Mechanism of inactivation of tobacco mosaic virus by x-rays, Nature, London *177*, 890

Lauffer, M A, and Wheatley, M, 1951, Destruction and denaturation of influenza A virus, Arch Biochem *34*, 436-447

Lea, D E, 1947, Actions of Radiations on Living Cells, New York, Macmillan

Levinthal, C., 1956, The mechanism of DNA replication and genetic recombination in phage, Proc Nat Acad Sc *42*, 394-404

Litman, R M, and Pardee, A B, 1956, Studies in mutagenesis, production of bacteriophage mutants by a disturbance of deoxyribonucleic acid metabolism, Nature, London *178*, 529-532

Longsworth, L G, 1942, Recent advances in the study of proteins by electrophoresis, Chem Rev *30*, 323-340

Loring, H S, 1938, Properties of the latent mosaic virus protein J Biol Chem *126*, 455-478

———, 1939, Properties and hydrolytic products of nucleic acid from tobacco mosaic virus, J Biol Chem *130*, 251-258

Loring, H S, and Schwerdt, C E, 1946, Isolation of a macromolecular constituent with properties of the Lansing strain of poliomyelitis virus, Proc Soc Exper Biol & Med *62*, 289-291

Luria, S E, Williams, R C, and Backus, R C, 1951, Electron micrographic counts of bacteriophage particles, J Bact *61*, 179-188

McClelland, L, and Hare, R, 1941, The adsorption of influenza virus by red cells and a new *in vitro* method of measuring antibodies for influenza virus, Canad J Pub Health *32*, 530-538

McLaren, A D, 1949, Photochemistry of enzymes, proteins, and viruses, Advances Enzymol. *9*, 75-170

Malkiel, S, 1947, Immunochemical studies on tobacco mosaic virus II The host-strain relationship J Immunol *57*, 43-49

Markham, R., Matthews, R. E F., and Smith, J D., 1954, Evidence for the existence of two types of ribonucleic acid, Nature, London *173*, 537.

Markham, R., and Smith, J D., 1951, Chromatographic studies of nucleic acids 4 The nucleic acid of the turnip yellow mosaic virus, including a note on the nucleic acid of the tomato bushy stunt virus Biochem J *49*, 401-407

Markham, R, and Smith, K. M., 1949, Studies on the virus of turnip yellow mosaic, Parasitology *39*, 330-342

Matheka, H D, and Armbruster, O., 1956, Die Anwendung von Ionenaustauschern zur Viruspräparation 2 Mitt Fraktionierung von Influenza-Virus Ztschr f Naturforsch *11b*, 193-199

Mattern, C F T, and du Buy, H G, 1956, Purification and crystallization of Coxsackie virus, Science *123*, 1037-1038

Miller, G L, Lauffer, M A, and Stanley, W M, 1944, Electrophoretic studies on PR8 influenza virus, J Exper Med *80*, 549-560

Miller, G L, and Price, W C., 1946, Physical and chemical studies on Southern bean mosaic virus. I Size, shape, hydration and elementary composition Arch Biochem *10*, 467-477

Miller, G L, and Stanley, W M, 1941, Derivatives of tobacco mosaic virus I Acetyl and phenylureido virus J Biol Chem *141*, 905-920

———, 1942, Derivatives of tobacco mosaic virus II Carbobenzoxy, -chlorobenzoyl, and benzenesulfonyl virus J Biol Chem *146*, 331-338

Morgan, C, Ellison, S A., Rose, H M, and Moore, D H, 1954a, Structure and development of viruses observed in the electron microscope I Herpes simplex virus J Exper Med *100*, 195-202

———, 1954b, Stucture and development of viruses observed in the electron microscope II Vaccinia and fowl pox viruses J Exper Med *100*, 301-310

———, 1955, Serial sections of vaccinia virus examined at one stage of development in the electron microscope, Exp Cell Research *9*, 572-578

Morgan, C., Howe, C., Rose, H M, and Moore, D H, 1956, Structure and development of viruses observed in the electron microscope IV Viruses of the RI-APC group J Biophys & Biochem Cytol *2*, 351-360

Narita, K, 1958, Isolation of acetylserylserylseryltyrosine from enzymatic digests of TMV-protein, Biochim et biophys acta, in press

Neurath, H, 1942, The investigation of proteins by diffusion measurements, Chem Rev *30*, 357-394

Neurath, H, and Cooper, G R., 1940, The diffusion constant of tomato bushy stunt virus, J Biol Chem *135*, 455-462

Neurath, H, and Saum, A M., 1938, The diffusion of tobacco mosaic virus protein in aqueous solution, J Biol Chem *126*, 435-442

Niu, C-I, and Fraenkel-Conrat, H, 1955, C-terminal amino acid sequences of four strains of tobacco mosaic virus, Arch Biochem *59*, 538-540

Niu, C -I, Shore, V, and Knight, C A, 1958, The peptide chains of some plant viruses, Virology, in press.

Northrop, J H, and Anson, M L, 1929, A method for the determination of diffusion constants and the calculation of the radius and weight of the hemoglobin molecule, J Gen Physiol *12*, 543-554

Oster, G, 1946, Molecular weights and other properties of viruses as determined by light absorption, Science *103*, 306-308

———, 1951, The isoelectric point of some strains of tobacco mosaic virus, J Biol Chem. *190*, 55-59

Oster, G, Doty, P M, and Zimm, B H, 1947, Light scattering studies of tobacco mosaic virus, J Am Chem Soc *69*, 1193-1197

Oster, G, and McLaren, A D, 1950, The ultraviolet light and photosensitized inactivation of tobacco mosaic virus, J Gen Physiol *33*, 215-228

Overman, J R, and Tamm, I, 1956, Equivalence between vaccinia particles counted by electron microscopy and infectious units of the virus, Proc Soc Exper Biol & Med *92*, 800-810

Pickels, E G, 1943, Sedimentation in the angle centrifuge, J Gen Physiol *26*, 341-360

Pirie, N W, Smith, K M, Spooner, E T C, and McClement, W D, 1938, Purified preparations of tobacco necrosis virus (Nicotiana virus II), Parasitology *30*, 543-551

Pollard, E, 1951, Ionizing radiation as a test of molecular organization, Am Scientist *39*, 99-109

Pollard, E C, 1953, The Physics of Viruses, New York, Acad Press

Price, W C, 1940, Thermal inactivation of four plant viruses, Arch ges Virusforsch *1*, 373-386

———, 1946, Purification and crystallization of Southern bean mosaic virus, Am J Bot *33*, 45-54

Putnam, F W, and Kozloff, L M, 1950, Biochemical studies of virus reproduction IV The fate of the infecting virus particle J Biol Chem *182*, 243-250

Randall, R, Mills, J W, and Engel, L L, 1947, The preparation and properties of a purified equine encephalomyelitis vaccine, J Immunol. *55*, 41-52

Reddi, K K, 1957, Structural differences in the nucleic acids of some tobacco mosaic virus strains I Monopyrimidine nucleotides in ribonuclease digests Biochim. et biophys acta *25*, 528-531

Reddi, K. K, and Knight, C A, 1956, Structural studies on the nucleic acids of some strains of tobacco mosaic virus I The residual material after ribonuclease digestion J Biol Chem *221*, 629-633

———, 1957, Terminal groups of tobacco mosaic virus nucleic acid, Nature, London *180*, 374-376

Ross, A F, 1941a, Purification and properties of alfalfa-mosaic virus protein, Phytopathology *31*, 394-410

———, 1941b, The determination of some amino acids in tobacco mosaic virus protein, J Biol Chem *138*, 741-749

———, 1942, The fractionation of the amino acids of tobacco mosaic virus protein, J Biol Chem *143*, 685-693.

Ross, A F, and Stanley, W M, 1938, The partial reactivation of formolized tobacco mosaic virus protein, J. Gen Physiol *22*, 165-191

———, 1939, The sulfur and phosphorus contents of tobacco mosaic virus, J Am Chem Soc *61*, 535-536.

Schachman, H K, and Lauffer, M A, 1949, The hydration, size and shape of tobacco mosaic virus, J Am Chem. Soc. *71*, 536-541.

Schäfer, W, and Zillig, W, 1954, Über den Aufbau des Virus-Elementarteilchens du Blassischen Geflugelpest, Ztschr f Naturforsch *9b*, 779-788

Schaffer, F L, and Schwerdt, C E, 1955, Crystallization of purified MEF-1 poliomyelitis virus particles, Proc Nat. Acad Sc. *41*, 1020-1023

Schramm, G, 1943, Über die Spaltung des Tabakmosaikvirus in niedermolekulare Proteine und die Ruckbildung hochmolekularen Proteins aus den Spaltstucken, *Naturwissenschaften 31*, 94-96

Schramm, G, and Bergold, G, 1947, Über das Molekulargewicht des Tabakmosaikvirus, Ztschr f Naturforsch *2b*, 108-112.

Schramm, G, Born, H J, and Lang, A, 1942, Versuch uber den Phosphoraustausch zwischen radiophosphorhaltigem Tabakmosaikvirus und Natriumphosphat, Naturwissenschaften *30*, 170-171

Schramm, G, Schumacher, G, and Zillig, W, 1955, Über die Struktur des Tabakmosaikvirus, Ztschr f Naturforsch *10b*, 481-492

Schwerdt, C E, and Fogh, J, 1957, The ratio of physical particles per infectious unit observed for poliomyelitis virus, Virology *4*, 41-52

Schwerdt, C E, and Loring, H S, 1947, The identification of three mononucleotides from tobacco mosaic virus nucleic acid, J Biol Chem *167*, 593-597

Schwerdt, C. E, and Schaffer, F. L, 1955, Some physical and chemical properties of purified poliomyelitis virus preparations, Ann New York Acad Sc *61*, 740-750

———, 1956, Purification of poliomyelitis viruses propagated in tissue culture, Virology *2*, 665-678

Scott, E M, and Lauffer, M A, 1946a, Thermal destruction of influenza A virus hemagglutinin III. The effect of urea Arch Biochem *11*, 179-184

———, 1946b, Thermal destruction of influenza A virus hemagglutinin IV The effect of initial virus concentration Arch Biochem *11*, 185-189

Shainoff, J R, and Lauffer, M A, 1956, Chromatographic purification of Southern bean mosaic virus, Arch Biochem *64*, 315-318

———, 1957, An application of ion exchange chromatography to the identification of virus activity with characteristic particles, Virology *4*, 418-434

Sharp, D G, Taylor, A R, Beard, D, and Beard, J W., 1942, Electrophoresis of the rabbit papilloma virus protein, J Biol Chem *142*, 193-202

Sharp, D G, Taylor, A R, McLean, I W, Jr, Beard, D, and Beard, J W, 1944a, Density and size of influenza virus A (PR8 strain) in solution, Science *100*, 151-153.

———, 1945, Densities and sizes of the influenza viruses A (PR8 strain) and B (Lee strain) and the swine influenza virus, J. Biol Chem. *159*, 29-44

Sharp, D G, Taylor, A R, McLean, I W, Jr, Beard, D, Beard, J W, Feller, A E, and Dingle, J H, 1944b, Isolation and characterization of influenza virus B (Lee strain), J Immunol 48, 129-153

Smadel, J E, Pickels, E G, and Shedlovsky, T, 1938, Ultracentrifugation studies on the elementary bodies of vaccine virus II The influence of sucrose glycerol, and urea solutions on the physical nature of vaccine virus J Exper Med 68, 607 627

Staehelin, M, 1957, Inactivation of TMV RNA with formaldehyde, Fed Proc 16, 254

Stanley, W M, 1935, Isolation of a crystalline protein possessing the properties of tobacco mosaic virus, Science 81, 644-645

———, 1936, The inactivation of crystalline tobacco mosaic virus protein, Science 83, 626-627

———, 1937a, Chemical studies on the virus of tobacco mosaic VIII The isolation of a crystalline protein possessing the properties of aucuba mosaic virus J Biol Chem 117, 325-340

———, 1937b, Chemical studies of the virus of tobacco mosaic IX Correlation of virus activity and protein on centrifugation of protein from solution under various conditions J Biol Chem 117, 755 770

———, 1938a, The isolation and properties of tobacco mosaic and other virus proteins, Harvey Lect Ser 33, 170-204

———, 1938b, Aucuba mosaic virus protein isolated from diseased, excised tomato roots grown in vitro, J Biol Chem 126, 125 131

———, 1939, The isolation and properties of tobacco ring spot virus, J Biol Chem 129, 405 428

———, 1940, Purification of tomato bushy stunt virus by differential centrifugation, J Biol Chem 135, 437-454

———, 1942a, The concentration and purification of tobacco mosaic virus by means of the Sharples super-centrifuge, J Am Chem Soc 64, 1804-1806

———, 1942b, The preparation and use of tobacco mosaic virus containing radioactive phosphorus, J Gen Physiol 25, 881-890

———, 1943, Chemical Structure and the Mutation of Viruses, in Virus Diseases, pp 35-59, Ithaca, Cornell Univ Press

———, 1944a, An evaluation of methods for the concentration and purification of influenza virus, J Exper Med 79, 255-266

———, 1944b, The size of the influenza virus, J Exper Med 79, 267-283

———, 1945, The preparation and properties of influenza virus vaccines concentrated and purified by differential centrifugation, J Exper Med 81, 193-218

Stanley, W M, and Anderson, T F, 1941, A study of purified viruses with the electron microscope, J Biol Chem 139, 325-338

Stanley, W M, and Lauffer, M A, 1939, Disintegration of tobacco mosaic virus in urea solutions, Science 89, 345-347

Stanley, W M, and Loring, H S, 1936, The isolation of crystalline tobacco mosaic virus protein from diseased tomato plants, Science 83, 85

———, 1939, Properties of purified viruses Relazioni del IV Congresso Internazionale di patologia comparata, 1, 45-82

Stanley, W M, and Wyckoff, R W G, 1937, The isolation of tobacco ring spot and other virus proteins by ultracentrifugation, Science 85, 181-183

Steere, R L, 1956, Purification and properties of tobacco ringspot virus, Phytopathology 46, 60-69

———, 1957, Electron microscopy of structural detail in frozen biological specimens, J Biophys Biochem Cytol 3, 45-60

Stent, G S, 1955, Decay of incorporated radioactive phosphorus during reproduction of bacteriophage T_2, J Gen Physiol 38, 853-865

Stent, G S, and Jerne N K, 1955, The distribution of parental phosphorus atoms among bacteriophage progeny, Proc Nat Acad Sc 41, 704-709

Taylor, A R, Beard, D, Sharp, D G, and Beard, J W, 1942, Nucleic acid of the rabbit papilloma virus protein, J Infect Dis 71, 110 114

Taylor, A R, Sharp, D G Beard, D, and Beard, J W 1943a, Isolation and properties of the equine encephalomyelitis virus (eastern strain), J Infect Dis 72, 31-41

Taylor, A R, Sharp, D G, Beard, D, Beard, J W, Dingle, J H, and Feller, A E, 1943b Isolation and characterization of influenza A virus (PR8 strain), J Immunol 47, 261-282

Taylor, A R, Sharp, D G, McLean, I W, Jr, Beard D, and Beard, J W, 1945, Concentration and purification of influenza virus for the preparation of vaccines, J Immunol 50, 291 316

Tiselius, A, 1937, A new apparatus for electrophoretic analysis of colloidal mixtures, Tr Faraday Soc 33, 524-531

———, 1947, Adsorption analysis of amino acid mixtures, Adv Protein Chem 3, 67

——— 1954, Chromatography of proteins on calcium phosphate columns, Arkiv kur Kemi 7, 443-449

Tiselius, A, Pedersen, K O, and Svedberg, T, 1937, Analytical measurements of ultracentrifugal sedimentation, Nature, London 140, 848 849

Vinson, C G and Petre, A W, 1929, Mosaic disease of tobacco, Bot Gaz 87, 14-38

———, 1931, Mosaic disease of tobacco II Activity of the virus precipitated by lead acetate Contrib Boyce Thomp Inst 3, 131-145

Williams, R C, 1953, A new method of freeze-drying for electron microscopy, Exp Cell Research 4, 188-201

———, 1957, The role of the electron microscope in virus research, in International Reviews of Cytology, vol 6, pp 129-191, New York, Acad Press

Williams, R C, and Fraser, D, 1956, Structural and functional differentiation in T_2 bacteriophage, Virology 2, 289-307

Williams, R C, and Smith, K M, 1957, A crystallizable insect virus, Nature, London, 179, 119-120

Williams, R C, and Wyckoff, R W G, 1944, The thickness of electron microscopic objects J Appl Phys 15, 712-716

Williams, R C, and Wyckoff, R W G, 1946, Applications of metal shadow-casting to electron microscopy, J Appl Phys *17*, 23-33
Wyckoff, R W G, 1949, Electron Microscopy, New York, Interscience
Wyckoff, R W G, Biscoe, J, and Stanley, W M, 1937, An ultracentrifugal analysis of the crystalline virus proteins isolated from plants diseased with different strains of tobacco mosaic virus, J. Biol Chem *117*, 57-71.
Zaitlin, M, 1956, The separation of strains of tobacco mosaic virus by continuous filter paper electrophoresis, Biochim et biophys acta *20*, 556-557

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3

Biochemistry of the Virus-Infected Cell

INTRODUCTION

A certain amount of biochemical information relevant to the mechanisms of virus multiplication has been available for many years However, the biochemistry of virus-infected cells as such has been studied for only a decade All earlier examinations of the biochemical effects of virus infection employed virus-infected tissue of higher animals and plants, and therefore the effects observed were not only a composite of normal behavior and the behavior of infected cells but also included the secondary effects which may occur as a result of the interaction of normal and infected cells in a milieu of the products of destroyed cells Secondary inflammatory changes were also an additional potential complicating factor in infected animal systems In such a situation the definition of the time relations of the stages of multiplication and of the primary effects of infection upon host cells in biochemical terms is almost impossible to achieve

It is instructive in this connection to examine the discussion which arose in the period of 1942 to 1946 between two capable groups of biochemists around the apparent metabolic effects of infection by poliomyelitis virus Since the energy sources of brain are predominantly derived from carbohydrate metabolism, and a number of the relevant enzyme reactions, such as those involved in anaerobic glycolysis, are studied readily, it seemed reasonable to determine if the capacity for anaerobic glycolysis in homogenates of brains of mice infected by poliomyelitis virus was altered significantly It was first reported that such homogenates showed a slightly inhibited (about 15%) anaerobic glycolysis (Racker and Kabat, 1942), and it was concluded that the virus may act by interfering with this metabolic activity of the nerve cell. Another group tried without success to repeat these results (Utter et al, 1945, Wood, 1944) Indeed, the latter worker concluded that since only a small portion of the brain cells was visibly damaged by infection, it was not likely that one could hope to reveal the potentially small difference in enzyme concentration in a homogenate of whole infected brain, even if the methods available for estimating enzyme activity and concentration were satisfactory Continuing investigation into this problem by this approach has revealed only a maze of complexities of experimental detail and difficulties in interpretation (Racker, 1954)

The problem of describing the biochemistry of normal tissue is difficult enough at best Modern biochemistry has had many successes in the isolation and the characterization of cell fragments and components, including such functional units as enzymes, but the discipline has had relatively few successes as yet in interpreting the behavior of intact functioning cells and tissues in biochemical terms. Nevertheless, in addition to the tasks of dissecting cellular structures and the intermolec-

ular reactions which are at the foundation of catalytic activity at a molecular level, the problem of interpreting the biologic activities of intact cells and tissues in terms of the organization and the activities of their molecular components is now recognized to be a major task of biochemistry. Therefore, we have entered consciously and determinedly into the area of cellular biochemistry, a branch of biochemistry well suited to the problems of virus disease, which for our present purposes may be considered primarily as cellular disease

In the light of these past experiences then, it can be seen that an essential prerequisite for the analysis of the biochemistry of viral infection had to involve the development of a methodology in which quantitative biology was the keystone How many host cells are we dealing with and what is their nutritional history? Are they all of one kind, i e, are they derived from a single clone? What proportion of the cells is infected, and when did this occur? When is virus released, and is this accompanied by cell destruction? These are the kinds of biologic information which we must have before we can obtain the most significant biochemical data.

Such controlled biologic systems may be afforded most readily (1) if relatively uniform populations of host cells derived from single cell lines may be grown separately in defined media and estimated as discrete units, (2) if purified virus preparations can be obtained, consisting predominantly of accurately and easily assayable infectious units, and (3) if infection can be established by a simple process of mixing cell and virus populations in such a way that the distribution of virus particles per cell may be calculated readily from the Poisson equation and confirmed by the differential estimation of free virus and infected cells.

The important conditions listed above for a controlled biologic system were first met for bacteria and certain virulent bacteriophages As a result of the pioneering investigations described in Chapter 7, the phenomenon of an infectious cycle was clearly demonstrated and analyzed

It was found that bacteria could be grown as separate cells under defined conditions and controllably infected with these phages The intensive study of a group of 7 phages, called T_1 through T_7, infecting the bacterium, *Escherichia coli*, was begun in the early 1940's by a small group of research workers who saw in this biologic system the opportunities for just such a rigorous quantitative biology which might permit a detailed analysis of the phenomena of virus multiplication and of mechanisms of viral inheritance. Infected cells remained intact for reproducible intervals, depending on the conditions of media, temperature, aerobiosis, etc After a period of virus multiplication the cells lysed, liberating many more virus particles than had been used originally to establish infection Electron microscopy revealed the characteristic morphology of the infecting virus particles and of the derived progeny at lysis (Luria et al, 1943). Purified concentrates of phage were obtained from lysates by differential centrifugation (Anderson, 1945), as described earlier by Schlesinger (1934, 1936) The chemical analysis of concentrates of the T_2 bacteriophage was begun (Hook et al, 1946, Cohen and Anderson, 1946) as an essential preliminary to the exploration of the metabolism of virus-infected cells, since it seemed reasonable to suppose that a knowledge of virus constituents might suggest the areas of metabolism in which the infected cell might be studied most profitably. The continuing analysis of this and related viruses has produced the most unexpected results—results which have been correlated with important biologic properties of the T_2 virus, and the virulence of the infections it produces The study of the multiplication of the T-even bacteriophages, i e., the T_2, T_4 and T_6 viruses, has been pursued in great depth, and it is now evident to most virologists that the major remaining problems with these systems primarily involve questions of the molecular architecture of virus structures and the interactions of these structures at molecular levels with a variety of bacterial components whose nature must also be understood at molecular levels Major biologic problems in these systems have been reduced to chemical and physical problems The chemists and the physicists, in taking advantage of the biologic tool which these systems make available, will not only obtain information relevant to mechanisms of virus multiplication but also will learn of hereditary units and the storage

of genetic information in molecular structure, of the transmission of genetic information and the duplication of structure, of the relation of genetic duplication to the development of cellular physiology and cellular pathology; and of many other basic problems of cellular biology. The fruits of investigation in this field have already provided an overwhelming demonstration of the importance of a sound quantitative methodology in the approach to biologic and biochemical problems

However, although it is clear that the biochemical study of bacteria infected by bacterial viruses is well developed, it must be noted that a comparable study of the biochemistry of animal viruses and their multiplication has barely begun It is only in the last 5 years that the task of analyzing the biology of animal virus multiplication at the cellular level has been assumed in a manner comparable with the approach used with the basteriophages The largest impetus for this development came from the demonstrations that, as in the assay of bacterial viruses, some animal viruses will produce plaques on monolayer tissue cultures (Dulbecco, 1952) and that these viruses will also grow in cell suspensions and in single cells (Dulbecco and Vogt, 1953) The first of these findings suggested that animal viruses might be assayed as accurately and as simply as the bacterial viruses, even as presaged earlier in experiments with the pox viruses (Beveridge and Burnet, 1946) The second result pointed not only to the possibility of an academic study of changes of mass and substance in infected cell suspensions but also augured the mass production of viral antigens for purposes of production of various viral vaccines The latter possibility has been brought to fruition in the development of various vaccines

However, with these systems it has been found that methodology alone is not sufficient to ensure progress The preparation of animal cells as suitable hosts is far more difficult, expensive and time-consuming than is the preparation of bacterial populations Satisfactory media are not easily definable and are less reproducible Animal viruses are not as readily available nor as easily assayable, they are often more unstable than the phages Infection in vitro is established far less efficiently, and virus yield is proportionately much less. Although great effort is being expended in attempting to overcome these experimental difficulties, progress has been disappointingly slow As a consequence, the biochemistry of animal virus infection is distinguished for the most part by the paucity of its data It will be the task of workers in this decade to bring our knowledge of the animal viruses up to the level of information available concerning bacterial virus multiplication Such an advance requires a great development of our knowledge concerning the nutrition, growth, genetics, biochemistry and physiology of animal cells grown in vitro, i e, tissue cultures In this instance, progress in animal virology will also provide great benefits for the future of cellular physiology and biochemistry

In the past several years much study has been given to the nutritional requirements of isolated mammalian cells (Stewart and Kirk, 1954, Eagle, 1955) and to their biochemical properties (Siminovitch and Graham, 1956; Siminovitch et al, 1957), to the conditions for developing clones from single cells from normal human tissues (Puck et al, 1956, Marcus et al, 1956) and to the cytologic and biochemical stability of such cell lines (Puck and Fisher, 1956) Such studies have been an essential preliminary to the development of optimal experimental systems for the study of viral multiplication For example, the effects of x-irradiation on carefully controlled human tissue cultures have been examined systematically, and it has been reported that cells damaged in this way become particularly susceptible to virus action (Puck and Marcus, 1956)

The requirements for advance in animal virology may call for the development of little-plumbed fields of biologic and chemical study For example, insect virus systems appear to provide the best instances of latency among animal virus systems Recognition of this opportunity has led to renewed effort on the cultivation of insect cells in vitro (Wyatt, 1956) In the systematic analysis of hemolymph as an approach to the development of nutritive media, it was found that, unlike the situation in mammalian sera, which contain free glucose, large amounts of phosphorylated sugars are normal constituents of hemolymph (Wyatt et al, 1956)

Although much is known of the properties of some isolated plant viruses (Chap 2), the analysis of their intracellular multiplication has advanced rather haltingly In large part this has arisen from the difficulties of assay and of establishing infection simultaneously in large numbers of plant cells. These problems of establishing suitable biologic materials have not yet been solved for plant virus systems Until they are, knowledge in this area will be highly unbalanced, and the plethora of information on plant virus structure can lead only to hypotheses whose definitive test will be difficult to achieve

Therefore, we are confronted with 3 levels of knowledge on the biochemistry of virus infection, the levels corresponding to the 3 main groups of viruses: virus-infected bacteria, animals, and plants With each group the state of available data has been determined roughly by the experimental biology developed in each area Although one approach to the presentation of these data could involve a systematic treatment of the known biochemistry of each virus type, we prefer to anticipate the development of a biochemistry of animal virus infection by discussing the kinds of experiments which have already been useful in analyzing infected cells. In so doing, although we shall draw heavily on the most advanced area, that of the bacterial viruses, it will be seen that the fields represented by the animal and plant viruses are not entirely barren.

THE CHEMICAL COMPOSITION OF VIRUSES

A detailed exposition of the chemical and physical properties of viruses is presented in Chapter 2 Several generalizations appear from this data which have been of considerable value in orienting studies on the bio-

tions, e g, the pox viruses, such as vaccinia virus, are larger than pleuropneumonia organisms Most viruses are larger than most proteins, although again it may be noted that a snail hemocyanin is larger than the virus of foot-and-mouth disease. This range of sizes suggests that most viruses possess a structural and chemical complexity which is less than that of cells and greater than that of a protein Until the present, the physical and chemical characterization of isolated virus particles has confirmed this. All viruses contain at least nucleic acid, in addition to protein However, whereas all cells contain two types of nucleic acid, deoxyribose nucleic acid (DNA), which is a characteristic component of chromosomes, and ribose nucleic acid (RNA), which is the only nucleic acid found in the cytoplasm of most cells, all known viruses appear to contain only one or the other type of nucleic acid The few exceptions to this rule appear to occur among the rickettsiae and the psittacosis-lymphogranuloma group of agents, some of which contain both types of nucleic acid and are increasingly being excluded from the "true" viruses for this and other reasons.

If viruses are smaller and chemically less complex than the cells they infect, it may be anticipated that they lack many other components essential to independent growth and multiplication, they probably will lack enzymes and numerous metabolic systems of their own, as indeed they do It can be supposed that these deficiencies define the parasitic dependence of a virus upon their host cells, which thereby are called upon to provide the essential metabolites and perhaps even essential enzymes for the synthesis of viral polymers and particles Therefore, it has been of interest to define in considerable detail the composition of virus particles and more particularly the structural and enzymatic deficiencies of these particles. If the formation of compounds which are present in a virus requires the participation of metabolites and enzymes which are absent from the virus but present in the cell, it is reasonable to believe that those particular aspects of host metabolism have been diverted to virus production and hence are of special interest.

Whether or not such a diversion need be sufficient to affect normal cell function may be approached at least superficially from a knowledge of the metabolic requirements of the normal cell and the extent of synthesis of virus components and virus in the infected cell However, such mass relations need not reflect the true relations of the diverted metabolism, since conceivably a diversion of a small percentage of host energy and product, occurring at a crucial site, may affect a critical portion of the host economy and cellular integrity. In variants of the tobacco mosaic disease, almost 70 per cent of the protein elaborated consists of virus protein, while only

mild stunting of the plant can be seen On the other hand, in tobacco ring spot disease, very small amounts of virus production are accompanied by cell destruction Nevertheless, in the search for key sites of cellular economy, it is important to know the extent of diversion of the infected cell to virus production

It may be noted in brief summary of the chemical data, that all viruses contain protein and one type of nucleic acid All known bacterial viruses contain DNA, and all isolated plant viruses contain RNA (Cohen, 1955). Until recently, all known insect viruses were found to contain DNA, and since some plant viruses are known to multiply in both plants and their insect vectors, the question of the nature of their nucleic acid presented an intriguing problem However, an insect virus has recently been found containing RNA (Zeros, 1956) The animal viruses may contain either RNA or DNA, thus, influenza virus contains RNA, and vaccinia virus and rabbit papilloma virus contain DNA Such studies have suggested that the nucleic acid metabolism of virus-infected cells might be particularly altered to favor the production of the type of nucleic acid characteristic of virus The value of such an educated guess concerning a potentially fruitful area of study is exemplified by studies of bacteria infected by phage in which a tremendous distortion of the pattern of nucleic acid metabolism was revealed, with the shift of synthesis occurring toward the formation of viral DNA (Cohen, 1947)

The analysis of fine structure of virus nucleic acids and proteins has been of considerable value in pointing to interesting metabolic experiments Three examples may be given. The DNA of T-even phages has been found to contain a unique pyrimidine, not known in the host bacterium (Wyatt and Cohen, 1953) As is described below, the problems of the role of 5-hydroxymethylcytosine, the origin of this compound and of the enzyme controlling its synthesis, appear to be central to the parasitism of the T-even viruses.

If the base compositions of the RNA of normal tobacco leaves and of tobacco mosaic virus are compared, it is found that the uracil content of virus RNA is almost twice that of the normal RNA (Reddi, 1956) Metabolic studies have revealed that the availability of uracil is limited during virus multiplication (Basler and Commoner, 1956) and uracil analogues, such as 2-thiouracil, have been most potent in inhibiting the multiplication of tobacco mosaic virus (Jeener, 1957).

Amino acid analyses have been recorded of the virus-producing polyhedral disease of the silkworm, and of the intimate developmental membrane and the capsular proteins of the virus (Bergold and Wellington, 1954) It has been shown that each of these structures produced in virus-infected cells may be distinguished by its content of amino acids From this, it may be inferred that the specific protein metabolism of the infected cell will vary as a function of the particular stage of infection under examination, and of the viral structure being produced at that stage

It has been reported that other structural components, such as lipids and polysaccharides, are contained in some viruses, such as ornithosis and influenza viruses In the first instance, since ornithosis virus is a member of the psittacosis-lymphogranuloma group, it may be asked if the term "virus" is appropriate In the second case, it should be noted that purified preparations of influenza virus contain antigens which cross-react with antisera to normal host components (Cohen, 1944, Knight, 1946) It is not known whether such normal antigens are simply admixed contaminants, adsorbed complexes, or exist as integral portions of the virus Therefore, it is not known if the lipid or polysaccharide of influenza virus are merely components of normal host antigen or are more intimately associated with virus duplication One approach to this problem could ask if the lipid and RNA of influenza virus have comparable origins in time in infected cells, a question which is readily answered with modern technics At the present time, with the exception of some unusual carbohydrate components of T_2 bacteriophage, it is not known that any constituents, other than nucleic acid and protein, reported to exist in viruses, are indeed involved in the duplication of virus substance

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activity, in addition to the presence of both DNA and RNA, a cellular morphology, and a sensitivity to certain antibacterial agents have served to eliminate these parasites from the category of the viruses. Most recently meningopneumonitis "virus" has been found to contain a DPNH-cytochrome C reductase (Allen and Bovarnick, 1957), an activity, which in conjunction with the large size of the agents of the psittacosis-lymphogranuloma group, their inhibition by antibiotics, and their complex growth cycle, has suggested their closer relation to the parasitic cells.

On turning to the true viruses, it should be noted that most assays for their possible enzymes were performed at a time (over 15 years ago) when only a limited number of systems, active in respiration, hydrolytic cleavages of various types, and some limited areas of carbohydrate metabolism could be determined In summary of this data, it may be said that no enzymatic component of an electron transport system has yet been observed in any plant, animal or bacterial virus, nor have any changes in the respiration of infected cells been noted to suggest the presence of a respiratory system in newly multiplied virus particles (Cohen, 1957; Gottschalk, 1957) However, vaccinia virus, although lacking the cytochromes and a variety of dehydrogenases, has been found to contain a coenzyme, flavin adenine dinucleotide, important in electron transport It must be asked, therefore, if in this virus a flavoprotein enzyme does not exist capable of carrying out some as yet undetermined reaction The added presence of copper and biotin in this virus poses the same question (Smadel and Hoagland, 1942)

Despite the apparent presence of these substances in vaccinia elementary bodies, tobacco mosaic virus and T_6 bacteriophage are not considered to contain significant amounts of the various coenzymes which embody biotin, pantothenic acid, riboflavin, folic acid, nicotinic acid and vitamin B_{12} (Sprince and Schoenbach, 1942; Kleinschmidt et al, 1956). The amount of acid-soluble phosphorus in these viruses is so low (1% or less of the total P) as to raise doubts concerning its reality as non-nucleic acid phosphorus In the absence of significant amounts of internal intermediary metabolites in the viruses, it has been suggested that the cell supplies the metabolites essential for viral polymer synthesis, since the viruses neither contain these substances nor have been found to be capable of any reaction which will provide essential metabolites or energy-rich compounds important in the fabrication of essential metabolites.

On the other hand, since the mechanisms of synthesis of polymeric nucleic acids and proteins are barely beginning to be clarified, it cannot be said with certainty that, given the essential metabolites, the viral polymers are incapable of catalyzing their own duplication. At the present time, the test of such a hypothesis cannot be designed nor can the appropriate metabolites be afforded.

As described in Chapter 2, the infectivity of various virus particles appears to reside in their nucleic acid components. To the extent that infection of host cells may be initiated by the nucleic acids alone, the problem of the synthesis of virus polymers must be posed in terms of (A) the polymer synthesizing enzymes of the host cells or (B) the possible activities of viral nucleic acids rather than viral proteins, or (C) the host enzymes and nucleic acid templates acting in unison to produce viral polymers or alternatively to produce new enzymes which are then capable of synthesizing the viral polymers. The problem of who does what in the fabrication of viral substance must be raised at 3 structural levels at least, that of (1) the small building blocks of the amino acids and nucleotides, (2) the polymeric viral proteins and nucleic acids and (3) the organization of the latter components to form the familiar isolated virus particles Although at the lowest structural level the responsibility of supply appears to rest with enzymes of the normal cell, we shall see certain instances in which key small building blocks are provided by enzymes of neither cell nor virus alone but rather by enzymes generated in the infected cells It is not certain that the intermediate levels of synthesis require enzymes in the sense in which they are commonly defined as catalytic proteins, and for tobacco mosaic virus at least the final assembly of nucleic acid and protein can occur as a nonenzymatic reaction (Chap 2)

Many hydrolytic systems are absent from the viruses Occasional reports of phosphatase activity, etc, have been attributed to contamination of the virus preparation under investigation However, an adenosine triphosphatase has been found to be intimately associated with the virus of avian myeloblastic leukosis (Mommaerts et al., 1954). Nevertheless, the significance of this association is obscured by the fact that the isolated virus particles contain normal chick antigens (Eckert et al, 1955a) Furthermore, the rates of thermal inactivations of enzyme and infectivity were significantly different, indicat-

ing at least the lack of identity of the centers for infectivity and enzyme activity (Eckert et al., 1955b). The role of the ATPase in the development of this virus infection is most obscure at present.

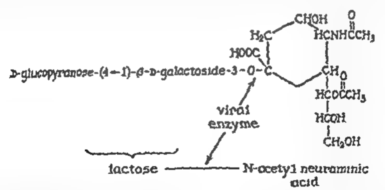
In two instances hydrolases, having important roles in the life cycles of the viruses, have been detected in virus particles (Gottschalk, 1957). As is described in Chapter 7, it has been shown that the tail of T-even bacteriophages contains an enzyme which helps to puncture the cell wall of *Escherichia coli*. Attachment of the virus to the bacterium exposes the viral enzyme which releases nitrogenous material from the cell wall (Barrington and Kozloff, 1956, Koch and Weidel 1956). Although it is suspected that the viral enzyme is a proteinase which cleaves peptide bonds, this has not yet been proved rigorously, since a well-defined substrate for the viral enzyme is not yet available.

Influenza virus also contains an enzyme which releases virus from its combination to erythrocyte surfaces after adsorption. The receptor at the red cell and other cell surfaces is thus considered to be the substrate for the viral enzyme. Better-defined substrates for this enzyme have been obtained, and these contain in each case a derivative of neuraminic acid, a compound which appears to be a basic unit contained in animal cell membranes. One such defined substrate is neuramin-lactose, possessing the following structure

In this interesting case, the study of the enzymatic activity of influenza virus has made important contributions to the analysis of the structure of animal cell surfaces and has created a bridge between the problem of virus growth and a host of other problems involving the structure and the function of cell surfaces.

COMPARATIVE ASPECTS OF VIRAL MULTIPLICATION

As the chemical and morphologic data indicate, the viruses are not a homogeneous group. It has been seen that many pl[illegible] [illegible] stru[illegible] chemical machinery to assist in the penetration of the well-protected plant cell. The penetration of new plants by virus may occur as a result of the infection by insects or after other abration of plant surfaces. On the other hand, the bacterial viruses have at their tail tip a single point of attachment to the bacterial receptors of the rigid cell wall. The interaction of cell wall and the viral tail tip in at least the T-even systems leads in sequence to the uncovering of a viral enzyme which opens a hole in the cell wall, extrusion of the viral tail spike, and injection of viral DNA into the bacterium. Unlike plant viruses



By cleaving the glucosyl linkage indicated above, the viral enzyme releases N-acetyl neuraminic acid or sialic acid from this and more complex substrates; therefore, it is termed a neuraminidase (Gottschalk, 1957). It has been suggested that the viral enzyme is not important in the penetration of the virus into the cell but rather is active in the escape of the virus through the cell surface

without any points of attachment or bacterial viruses with a single point of attachment, many animal viruses possess multiple points of attachment to cell receptors. This is revealed by the phenomenon of hemagglutination in which a virus particle may serve as a bridge connecting erythrocytes or in some instances separated host cells in tissue cultures

(Scherer, 1952) Thus, although a single antibody molecule is sufficient for the inactivation of Newcastle disease virus, such inactivated virus particles can adsorb to host cells. Several such molecules are required to prevent adsorption of the virus to the host (Rubin and Franklin, 1957). After attachment of the virus particle the surface membrane of the animal cells is considered to be capable of enveloping the virus particle and fulfills an active role quite unlike that of the corsetlike plant or bacterial cell wall.

The liberation of various viruses from infected cells also appears to vary as a function of the surface structure of the various types of host cells involved. Thus, the removal of a plant virus from a plant requires the rupture of the tough plant cell walls by the penetration of insect mouth parts or of a parasitic plant such as dodder. Spread of virus within the plant may be effected by intracellular plasmodesmata, thereby obviating the need of an additional rupture of the cell wall in passing from one cell to another. However, in bacterial virus systems, virus liberation always involves lysis of the cell wall and cell destruction. Very little is known about the mechanisms of this phenomenon. In animal virus systems, several mechanisms appear to be operative. Some workers have postulated the spread of virus from one cell to another by intracytoplasmic threads. In other cases, notably influenza virus systems, virus extrusion is clearly associated with the development of long virus-containing filaments on the surface membrane and their liberation without cell rupture. Unlike the bacterial viruses in which the liberation of all phages from a single cell occurs simultaneously with cell lysis, the liberation of various animal viruses from a given cell may be a slow process taking many hours (Henle, 1953, Dulbecco and Vogt, 1954; Rubin et al., 1955). In other cases, the development of plaques in tissue cultures infected by poliomyelitis virus, vaccinia virus, herpes virus, etc., indicates the spread of infection at least in part by a lytic mechanism of release as in the bacterial virus systems. However, it is by no means evident that the intracellular spread of any of these animal viruses occurs in vivo predominantly as a result of a lytic release.

The heterogeneity of the viruses is also clearly revealed in the analysis of the patterns of viral parasitism, which extend from systems in which infection leads almost exclusively to the synthesis of virus to those in which infection permits both virus and host cell to multiply together. In the former pattern, best exemplified by the effect of the T-even phages on the host *E. coli*, strain B, the nuclear apparatus is destroyed, and the metabolism of the host is diverted to the synthesis of large amounts of specific virus substance. Virus particles accumulate within the cell and are liberated by lysis. In the latter pattern, exemplified by the effect of phage lambda which infects *E. coli*, strain K_{12}, the virus may, depending upon environmental conditions, take a virulent path like that of the T-even phages, or alternatively, in a temperate cycle associate its genetic apparatus with that of the host cell and multiply in unison with it. In certain instances this symbiotic or lysogenic relationship may be disrupted by external stimuli, such as treatment by ultraviolet radiation or carcinogens. The phage then begins a maturation and uncontrolled multiplication leading to the accumulation of virus particles and cell lysis.

In these systems virulence leading to cell lysis produces an extracellular phase of virus existence in which the hereditary determinants of the phages are protected by a tough protein coat resistant to the enzymes, the proteases and the nucleases, which are liberated by lysing bacteria. In addition, as noted above, this coat is also highly differentiated for purposes of transferring the nucleic acid charge of the virus through the tough bacterial cell wall and is shed from the genetic material prior to its intracellular multiplication. Similarly, the protein coat of a lysogenic phage is not found during its reproduction as a prophage on the bacterial chromosome, or as vegetative phage after induction. Thus, these differences in the patterns of virulent and temperate infection greatly influence phenomena of protein synthesis.

In the bacterial viral systems the hereditary determinants of the virus are DNA and therefore are similar to nuclear determinants. This identity may be taken to suggest that in these systems we are dealing with aberrant nuclei or chromosomes. Certainly virulent phage multiplication results in the degradation of

the bacterial nucleus and DNA (Luria and Human, 1950). In plant virus systems, it appears that the RNA of the virus is the genetic material which is also shed from its protein coat Indeed, it is possible that, in the transmission of infection within a plant by intracytoplasmic bridges, the free RNA of tobacco mosaic virus may be the predominant unit of infectivity Since the plant viruses are found mainly in the cytoplasm and in containing RNA exclusively, more nearly resemble cytoplasmic units, it has been suggested that these viruses perhaps arise as aberrant cytoplasmic units, as in the variegation of plastids In the plant virus systems then, the metabolic alterations which might be explored initially and most fruitfully might be those expected in a cytoplasmic disease Thus, if electron microscopy suggests that beet yellows virus may originate in a diseased chloroplast (Leyon, 1953), one might explore the photosynthesis of normal and diseased cells and chloroplasts, as well as their synthetic capabilities toward RNA and protein

How shall we look at the animal viruses? Most of the known insect viruses produce nuclear inclusions and contain DNA It can be anticipated that the effects of infection in these systems will be quite different from those of the RNA-containing insect virus which produces cytoplasmic polyhedrosis

The study of infectious viral papilloma of human skin has also led to the observation of intranuclear DNA-containing inclusion bodies This in turn pointed to the desirability of a cytochemical study of DNA and histone production in infected cells at different developmental stages (Bloch and Godman, 1957) Many of the adenoviruses produce intranuclear inclusions which contain crystalline arrays of DNA-containing particles (Morgan et al, 1956, Bloch et al, 1957, Boyer et al, 1957) The existence of such particles points to DNA metabolism as a critical area of study in infection by these viruses

Briefly then, determination of the sites of virus synthesis and multiplication should be quite useful in orienting biochemical work In some instances, localization of these viruses within the cells can pose certain problems quite sharply For example, vaccinia virus which contains DNA appears to reproduce in the cytoplasm. Then it may be asked if this virus contains a system capable of synthesizing deoxyribose polynucleotides or are polynucleotide fragments of this nucleic acid preformed in the cell nucleus and then transferred to the cytoplasm where the virus organizes these pieces into virus DNA (Gaylord and Melnick, 1953, Morgan et al, 1954)

However, it cannot be supposed that existing data are sufficient in all virus diseases to orient the biochemical approaches to the infected cell For example, viruses of the influenza group are now known to contain RNA, antigens related to cytoplasmic constituents, and are often liberated in surface filaments It might be supposed that these viruses are entirely cytoplasmic in origin However, striking studies by means of fluorescent antibodies on production of viral antigen have revealed extensive nuclear involvement in the production of the nucleoprotein antigen of influenza and fowl plague viruses (Watson and Coons, 1954, Breitenfeld and Schafer, 1957). DNA changes in the nuclei of influenza virus-infected cells have also been reported, thereby implicating this substance as well in the multiplication of an RNA virus (Wolff, 1957) Nuclear and DNA changes have been observed recently in poliomyelitis-infected cells as well (Tenenbaum, 1957) It is clear then that the elucidation of the cellular site of virus multiplication is often a problem in its own right whose solution is useful in the evolution of a suitable cellular orientation to the biochemistry of a specific virus infection

METHODOLOGY IN THE STUDY OF INFECTION

Obviously, the elucidation of biochemical events in the course of infection may be accomplished by the chemical analysis of the changes in the components in infected cells Until the present, such an approach has improved only slowly in the chemical finesse and delicacy of its dissection, since this type of problem has not yet attracted many skilled biologically oriented chemists In lieu of this type of direct approach, biochemical information may be obtained less directly by studying the effects of environmental change on the biology of virus production When, in addition, either of these approaches is combined with morphologic analysis, as performed by skillful electron microscopists, the derivable

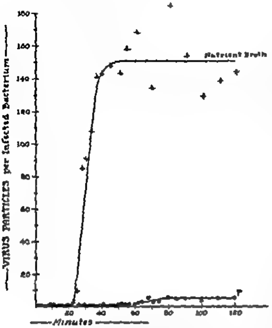


FIG 19 One-step growth curves for T2+ bacteriophage in broth-grown bacteria in broth and synthetic media (F) (Fowler, C B, and Cohen, S S, 1948, Chemical studies in host-virus interactions IV A method of determining nutritional requirements for bacterial virus multiplication, J Exper Med 87, 259-274)

biochemical information is accorded an additional dimension. It is precisely in the field of virology, which is concerned with genetic duplication at molecular and intermolecular levels, that chemical dimension meets biologic dimension most clearly, in this field, electron microscopy, even in its current development, becomes a tool for the analysis of both chemical and biologic structure

It is immediately evident that a study in which detectable chemical changes are correlated with both biologic and morphologic data will provide the best information on the course of virus multiplication Such a study depends on the development of methods for opening cells to liberate biologically significant units. In the field of the bacterial viruses, a number of technics have been devised to break open infected cells. One of the earliest of these used cyanide to provoke autolysis and liberation of virus and revealed that intact virus particles could not be observed in infected cells until about the second half of the latent period of multiplication (Doermann, 1948; 1952). A joint examination of DNA synthesis and the appearance of intact virus in infected bacteria demonstrated that several minutes intervened between the two functions, i e, that the formation of DNA did not complete virus formation (Cohen, 1949). Subsequent analyses of the kinetic relations of the newly synthesized DNA and the intact virus particles have been performed by Hershey (1953) and are discussed in some detail in Chapter 7.

In the field of animal viruses, a number of workers have studied the relationship of the

particles The appearance of these newly formed biologically active units poses the problem of their definition to the chemists. *Examples of these kinetic studies are presented in later chapters*

From these types of analysis, which are in their infancy, a general pattern of virus multiplication has emerged which is typified by that observed in bacterial virus systems Biologically unique components of virus are found before intact virus particles and indicate the existence of terminal steps of assembly after a long series of synthetic reactions Unlike the multiplication of cells in which the cell maintains its integrity and capacity for multiplication throughout its life cycle, the viruses

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CHEMICAL EFFECTS ON VIRUS MULTIPLICATION AND YIELD

In the approaches to the biochemistry of viral-infection to be considered immediately below, the number of infectious virus particles and the rate at which they are produced are used as a biologic measure of biochemical processes. In a few studies, it will be seen that changes in the quality of the particles produced are also sought and detected, as in an increased production of mutations or otherwise modified or deficient particles

NUTRITIONAL REQUIREMENTS

The environment essential for the multiplication of any virus consists of the interior of a cell, which in turn will tend to be modified by variations in the external environment of

the cell Only the external environment can be varied controllably and directly, and this can be done to affect not only the environment in which the virus is produced but the uninfected host cell as well. Under certain conditions it is possible to alter the enzymatic complement [redacted]f the uninfected host cell, creating deficiencies which may be used to explore requirements for virus multiplication A more common alternative approach is to vary the environment surrounding the infected cells. Examples of results obtained by both technics will be given

Two basic experiments with bacterial virus systems exemplify the types of results to be obtained Bacteria, e.g , *E. coli*, may be grown in broth, which contains a wide variety of metabolites, and minimizes the necessity of the cell to develop enzymes for the production of many of these compounds When such enzymatically deficient bacteria are resuspended in media containing broth, or mineral salts and a simple compound such as lactate or glucose as sole carbon source, it [redacted] found that on infection in these media, broth supports a far greater production of virus in a shorter time than does the simple medium (Cohen, 1949) As shown in Figure 19, cells grown in broth are poorly equipped to produce virus in a medium containing lactate as sole carbon source It may be inferred that in this system the cell uses many exogenous metabolites in the synthesis of virus components Supplementation of the lactate medium with an amino acid hydrolysate plus nucleic acid derivatives permits virus multiplication to about the level supported by nutrient broth (Fowler and Cohen, 1948)

Given a completely defined medium which can support a high level of virus multiplication in broth-grown bacteria, it [redacted] now possible to determine the effect of omitting single constituents, as in Figure 20 It can be seen from this experiment that exogenous tryptophan and leucine are important for T_2r^+ virus multiplication in this system. Further-

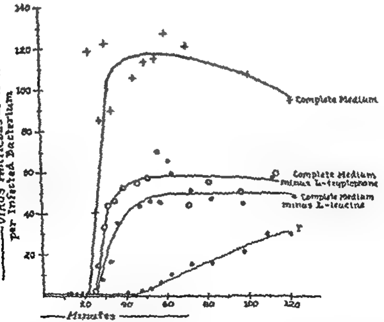

FIG 20 One-step growth curves for T2r+ in broth-grown bacteria in media of different compositions (Cohen, S S , and Fowler, C [redacted], 1948, Chemical studies in host-virus interactions V. Some additional methods of determining nutritional requirements for virus multiplication, J Exper Med *87*, 275-282)

more, it can be shown by chemical estimation that the synthesis of virus nucleic acid is inhibited under conditions of such restriction of single amino acids (Cohen and Fowler, 1948)

In extending this approach to animal virus systems, it has been found that tissue cultures of minced chicken embryos maintained in inorganic salts and glucose for up to 15 days can then be infected with psittacosis virus without virus production Supplementation of these cultures by a mixture of amino acids, nucleic acid derivatives and other compounds permits the elaboration of virus As seen in Figure 21, if selected single components, such as phenylalanine or tryptophan, were omitted from the complex mixture, virus multiplication did not occur (Heggie and Morgan, 1956). The development of synthetic media for the growth of animal cells and the production of virus in such cells have already permitted the demonstration of the requirements for exogenous glucose and glutamine in the optimal production of poliomyelitis virus in human cells, such as the HeLa cell (Eagle and Habel, 1956)

Effects of Inhibitors

Various inhibitors and antimetabolites have been found to be useful, not only in imposing deficiencies on infected cells, but they furnish greater potentialities for the dissection of the multiplication cycle than do the simple nutritional technics. Thus, it is possible to infect in the presence of an inhibitor whose action can be reversed at some later interval by the addition of an appropriate metabolite. Alternatively, the inhibitor itself may be used to interrupt later stages of multiplication which can then be restarted with metabolites In such experiments, a careful study of virus production and liberation will reveal whether the inhibitor does indeed affect a process essential to virus multiplication during the period in which the infected cell was exposed to its action. Such studies with inhibitors relate not only to the apparently academic problem of understanding details of the infectious cycle of a virus but are evidently of considerable interest in developing a chemotherapy of viral infection.

The use of citrate to bind calcium ions has

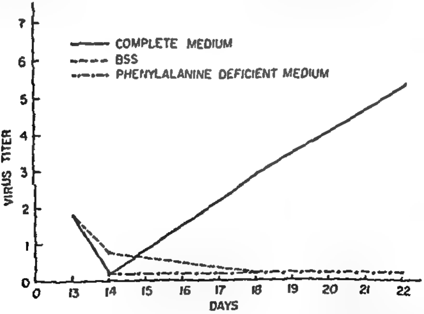

Fig. 21 The effect of phenylalanine deficiency on the stimulation of growth of psittacosis virus in chick embryo tissue culture cultivated in [illegible]

been used to reveal a role for these ions in the adsorption of some bacterial viruses in bacteria. Calcium is also found to be important in early stages of phage multiplication, as summarized by Adams (1954) Similarly, potassium has been found to be important in several stages of multiplication of influenza virus (Levine et al, 1956) Earlier it had been shown that the inhibitor, α-amino-α (p-methoxyphenyl) methane sulfonic acid, affected a very early stage of viral multiplication, as shown in Figure 22 (Ackermann and Maassab, 1954) It was then shown that such an inhibition may be reversed by potassium ions (Levine et al., 1956)

The effects of specific amino acid analogues were first clearly demonstrated in bacterial virus systems It had been shown that 5-methyl tryptophan does not inhibit T-even phage adsorption but does block virus multiplication (Cohen and Anderson, 1946, Fowler and Cohen, 1947) As shown in Figure 23A addition of the analogue in the first half of the latent period completely prevents virus liberation Indeed, when infected cells are held in the presence of the agent they slowly and irreversibly lose the ability to produce virus, a point potentially of interest in the chemotherapy of infection On the other hand, if the agent is added in the second half, multiplication is inhibited, but virus already produced intracellularly is liberated. In Figure 23B it can be seen that the subsequent addition of tryptophan to the cells inhibited at the inception of infection permits the development of a normal period of virus multiplication. Such an experiment shows that the analogue prevents processes requiring tryptophan, presumably protein synthesis, at the very beginning of multiplication It has been shown with this agent and other inhibitors, such as chloramphenicol, as well as in experiments with deficient mutants, that such early protein synthesis is essential to the later synthesis of viral DNA (Cohen, 1947, Burton, 1955, Tomizawa and Sunakawa, 1956, Hershey and Melechen, 1957) Furthermore, as can be seen in Figure 23C, if multiplication is interrupted by 5-methyl tryptophan and restarted by the addition of tryptophan, the processes take up exactly where they left off In this way requirements for an amino acid can be demonstrated throughout various periods of virus multiplication

In a similar way, the effects of compounds such as p-fluorophenylalanine and methoxinine

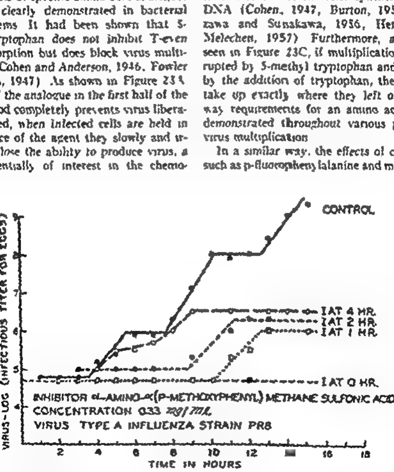

FIG 22 Effect of an inhibitor on the latent period of influenza virus multiplication. (Ackermann, W W, and Maassab, H. F., 1954, Growth characteristics of influenza viruses the influence of sulfonic acid, J Exper Med *99*, 105-117)

more, it can be shown by chemical estimation that the synthesis of virus nucleic acid is inhibited under conditions of such restriction of single amino acids (Cohen and Fowler, 1948)

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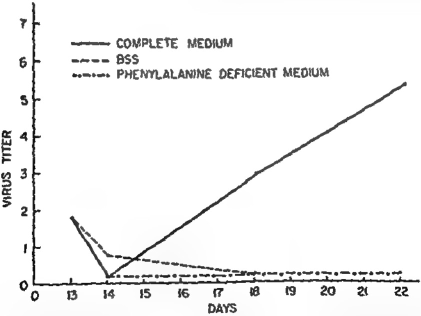


Fig. 21. The effect of phenylalanine deficiency on the stimulation of growth of psittacosis virus in chick embryo tissue culture cultivated in basal salt solution (BSS) for 13 days. (Heggie, A. D, and Morgan, H. R., 1956, Latent viral infection of cells in tissue culture. III. Role of certain amino acids, Proc. Soc. Exper. Biol & Med., 92, 506-509)

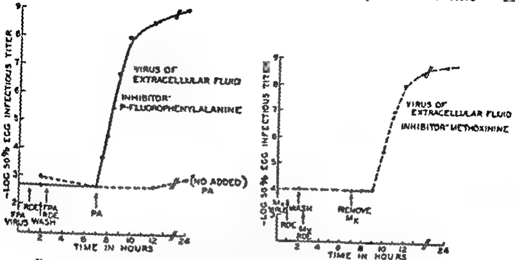


FIG 24 (A, *Left*) The production of the PR8 strain of Type A influenza virus in chorio-allantoic membrane after release of inhibition of p-fluorophenylalanine (FPA) by addition of phenylalanine (PA) The infected tissue was washed with receptor-destroying enzyme (RDE) to prevent readsorption of released virus (B, *Right*) The production of influenza virus after removal of inhibitory methoxinine (Mx) by washing the infected tissue (Ackermann, W W, and Maassab, H F., 1955, Growth characteristics of influenza virus II Biochemical differentiation on stages of development J Exper Med *102*, 393-402)

have been studied on influenza virus multiplication in isolated pieces of chick chorio-allantoic membrane (Ackermann and Maassab, 1955) As shown in Figure 24A, addition of phenylalanine to release the inhibition produced by fluorophenylalanine permits an immediate rapid release of virus, indicating that the inhibitor permits certain essential reactions to continue On the other hand, as shown in Figure 24B, the release of the inhibition produced by methoxinine requires a lag of at least 2 hours before virus release It is evident that this compound is acting more like 5-methyl tryptophan and affects virus multiplication at a point different from that affected by fluorophenylalanine

In studying the inhibition of influenza virus and vaccinia virus by benzimidazole derivatives, it was shown that for the former virus, the most potent inhibitor was a N_1-β-D-ribofuranosyl benzimidazole (Tamm et al , 1956) Such a nucleoside is most likely to affect the production of RNA, the kind of nucleic acid found in the virus On the other hand, in inhibiting the multiplication of vaccinia virus, a DNA virus, the benzimidazole ribosides were only as active as the free benzimidazoles, implying that important aspects of nucleic acid metabolism involved in the synthesis of this virus are different from those observed in the multiplication of influenza virus

Many compounds are now available for the study of the inhibition of virus multiplication Their structures and modes of action have been discussed extensively by Matthews and Smith (1955).

INHERITANCE AND MUTAGENESIS

As discussed in Chapter 2, it is now believed that the information essential to the duplication of viral structure is mainly, if not entirely, coded within the structure of the nucleic acids of the viruses Such a relation of nucleic acid to inheritance was first demonstrated in the transformation of inherited characteristics of pneumococci by means of isolated bacterial DNA, a similar relation of DNA to inheritance has since been extended to many micro-organisms Since the nucleic acids of the T-even bacterial viruses and of tobacco mosaic virus and its strains are infectious and can transmit specific genetic characters of the respective viruses, these substances appear also to embody the viral genetic determinants The isolation of in-

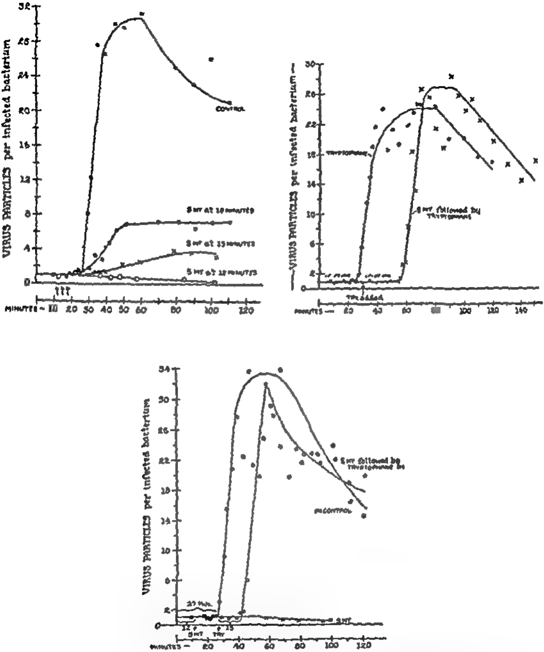


FIG 23. (A, *Top, left*) Inhibition of T2r+ virus production by the addition of 5-
. . . . the latent period. (B, *Top, right*) Inhibition of 5-methyl
= tryptophan (LP-latent period) (C, *Bot-*
-rupted by 5-methyl tryptophan (Cohen,
dies on host-virus interactions III Trypto-
multiplication in the *Escherichia coli*-T2
bacteriophage system, J. Exper Med 85, 771-784)

the liberation of T_2 particles. This is interpreted to signify that in mixed infection DNA *characteristic of T_2 may add an adsorptive* tail possessing T_4 proteins. After such phenotypic mixing (Novick and Szilard, 1951), the next generation obtains proteins determined by the T_2 nucleic acid. Thus, the assembly *process is in part conditioned by the environment* of proteins provided within the host cell. This and other host-induced modifications of the viruses have been discussed by Luria (1953).

Hereditary modifications of viruses have not been effected by various treatments of the isolated particles alone. However, such mutations may be made to occur in relatively high frequency by ultraviolet radiation of both virus and host bacteria (Weigle, 1953, Dulbecco, 1953). Jacob (1954) has observed the *increase of mutants by preirradiation of the* host bacterium prior to infection by normal virus. It is evident that derangements of host metabolism can markedly affect the quality of the virus nucleic acid produced.

Treatments of phage-infected bacteria with proflavine (DeMars, 1953) or streptomycin (Fernandez et al., 1953) also increased the percentage of mutations in the emergent progeny. *These agents did not appear to select* the mutants but did induce their formation. The mechanisms of these mutagenic actions are not known, nor have the mutagenic effects themselves been extended to plant or animal virus systems.

In recent years it has been demonstrated that unnatural synthetic purine and pyrimidine bases can be used for the synthesis of viral nucleic acids. Thus, 5-bromouracil will replace thymine in the synthesis of bacteriophage DNA. A number of compounds, including 2-thiouracil in place of uracil, 8-azaguanine in place of guanine, etc., can be inserted into the ribose nucleic acid of tobacco mosaic virus particles.

In the former case, thymine-deficient bacteria may be grown in thymine and infected *with T_2 bacteriophage in the presence of* bromouracil. The emergent progeny contain a very high percentage of inactive particles (about 80%). However, within the population of active particles is found DNA in which *all of the thymine is replaced by bromouracil,* and among such active particles may be found a high percentage of mutants (Litman and Pardee, 1956). Their changed genetic constitution is maintained in subsequent generations, although the unnatural bromouracil is replaced in turn by thymine.

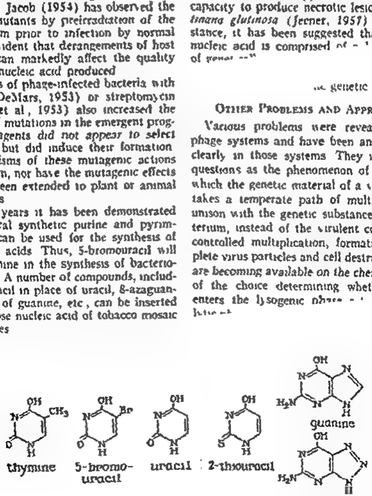
Thiouracil represses the production of tobacco mosaic virus. However, whatever virus is produced contains some of the unnatural base but does not appear to have a lowered capacity to produce necrotic lesions on *Nicotiana glutinosa (Jeener, 1957). In this in*stance, it has been suggested that the virus nucleic acid is comprised of ... of ...

... genetic units.

OTHER PROBLEMS AND APPROACHES

Various problems were revealed first in phage systems and have been analyzed most clearly in those systems. They include such questions as the phenomenon of lysogeny in which the genetic material of a virus particle takes a temperate path of multiplication in unison with the genetic substance of the bacterium, instead of the virulent course of uncontrolled multiplication, formation of complete virus particles and cell destruction. Data are becoming available on the chemical nature of the choice determining whether a virus enters the lysogenic phase ...

... latency



thymine 5-bromouracil uracil 2-thiouracil guanine 8-azaguanine

fectious nucleic acids from virus-infected animal cells has also been reported Indeed, from the point of view of the analysis of the chemical nature of the transmission of the genetic information by isolated nucleic acids, the viral systems may be more suitable than bacterial systems, since the isolated viral nucleic acids are biologically, and presumably chemically, more homogeneous than are the bacterial nucleic acids, i e, they contain many fewer genetic determinants

In all of these studies, the demonstration that a portion of a virus contains such genetic properties is biologic. Cells capable of virus production are exposed to the action of the nucleic acid, and the liberation of virus is detected by indicator host cells In this way when the RNA of one strain of tobacco mosaic virus is combined with the protein of another strain and such reconstituted particles are used to infect a cell, it can be detected that the viral progeny possess the biologic characteristics of the strain that provided the nucleic acid

Until recently, the evidence that the DNA

phage DNA from phage into the cell was demonstrated This is considered in greater detail below However, it proved to be very difficult to show that significant amounts of protein were not also injected into the cell The polymeric DNA of the T-even phages will not penetrate into intact *E. coli* (Cohen, 1947) However, penetration appears to be considerably facilitated by the use of bacterial protoplasts, i.e , bacteria denuded of their cell wall by enzymes and preserved within a naked membrane by hypertonic media Phage particles, disrupted to release their DNA, at least partially, which are inactive in infecting intact bacteria, are reported to be capable of establishing infection in protoplasts (Spizizen, 1957; Fraser et al., 1957) Such capabilities of disrupted particles are destroyed by deoxyribonuclease (DNAase), although this enzyme does not affect intact virus Surprisingly, the infectivity is also destroyed by trypsin, implicating peptide bonds in the infectious principle.

The appearance from infected cells of viruses differing in some inheritable characteristic from the infecting parent may be taken as evidence of some alteration in the composition and the structure of the nucleic acid of the progeny Thus, experiments on mixed infection of one cell with several viruses have revealed the development of individual progeny containing characteristics of the several viruses Several such types of exchange are known and reflect the several ways in which the information derived from the nucleic acid of the several viruses can be used to make a single particle. In experiments in T-even phage systems, it has been observed that from 15 to 25 essential exchangeable genetic units exist within these viruses (Luria, 1947, Delbruck and Bailey, 1946; Doermann et al, 1955) It may then be inferred that various parts of the DNA charge of the virus are biologically and therefore chemically distinct, although conceivably bound in a single chemical unit. The physical bases of such exchange are obscure at present; it is not known whether the exchange involves the exchange of bits of infecting DNA prior to or during multiplication or if the exchange occurs during assembly of the different kinds of units of DNA elaborated in the infected cell.

An in vitro reshuffling of the parts of a viral nucleic acid has been claimed for the nucleic acid of tobacco mosaic virus. Commoner et al (1956) have isolated the nucleic acid of this virus and have combined it with virus protein to give infectious nucleoprotein Such reconstituted particles were then found to give rise to an unusual number of mutant strains. In addition, fractionation of the reconstituted nucleoprotein and comparison with the fractionation of the original nucleoprotein have revealed marked differences in the two populations of particles

Genetic recombination technics in phage-infected bacteria have been used to calculate the linear order of determinants within the linear phage DNA One such study has given rise to the estimate that the chemical unit

controlled by separate genetic determinants which are many recombination units apart, suggesting that these structural features of the tail arise from differences in protein structure controlled by different genetic units (Brenner, 1957)

In experiments in which *E coli* is infected with a mixture of T_2 and T_4 viruses, progeny possessing characteristics of T_4 have been obtained, i.e., they infect cells resistant to T_2 However, occasionally infection with such T_4-like progeny leads in the next generation to

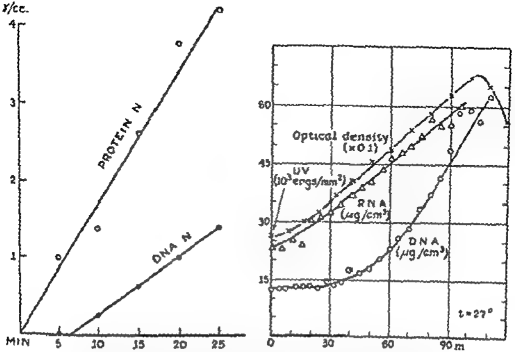


FIG 26. (A, *Left*) The synthesis of protein and the nucleic acids in T2-infected *Escherichia coli* (Cohen, S S, 1947, The synthesis of bacterial viruses in infected cells, Cold Spring Harbor Symp Quant Biol *12*, 35-49) (B, *Right*) The synthesis of the nucleic acids in lysogenic *B megatherium* after induction with ultraviolet light (Siminovitch, L, 1953, Biochemical modifications of the bacterial host during bacteriophage development, Ann Inst Pasteur *84*, 265-273)

cation have now been fulfilled, no experiments of this character have yet been reported

EFFECTS REVEALED BY CHEMICAL ANALYSIS

Changes of Mass. Given virus-infected cells, the chemical methods which must be employed to detect changes of substances will depend on the quantities involved as well as on the kinds of substance to be examined Under favorable conditions virus-infected bacteria may liberate as many as several hundred virus particles, the dry weight of each of which is of the order of 0 1 per cent of the host cell It is evident then that an infected bacterium (*E coli*-T_2) may synthesize as much as 25 per cent of its mass in virus-specific products However, the changes in constitution of the main classes of intracellular substance will reflect the formation of viral components only if such synthesis is net synthesis, i e, if host substance is degraded and resynthesized to viral material of the same general class, it may not be possible to observe gross changes in the chemical composition of infected cells In the *E coli*-T_2 system, changes are readily detectable by gross analyses T_2-infected cells cease multiplying but continue to synthesize protein at a constant rate The synthesis of DNA stops briefly and then resumes at a rate of several fold (4 and more) that observed in uninfected cells. The RNA content of infected cells remains essentially constant (Cohen, 1947) This point will be considered in greater detail below where it will be shown that the apparent constancy of quantity masks considerable metabolic activity These phenomena are represented in Figure 26A

The changes in components of infected cells can be studied under conditions of abortive infection with damaged virus particles or under the influence of inhibitors, such as proflavin or 5-methyl tryptophan As noted earlier, inhibition of protein synthesis with

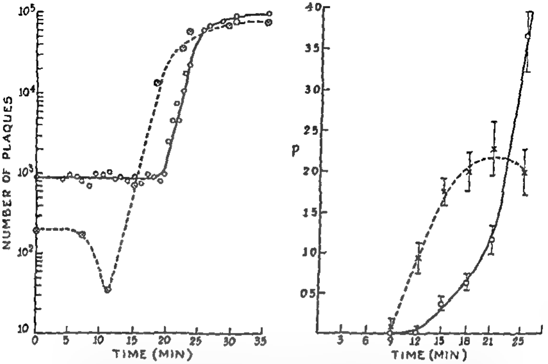


FIG. 25 (A, *Left*) Intracellular growth of T4r phage Small circles are plaque counts of material plated from the culture. Circles with crosses are counts after decompression breakage (B, *Right*) Growth curve of doughnuts (dotted line) and phage (solid line) P-1 means 15 particles per bacterium. (Levinthal, C, and Fisher, H, 1952, The structure development of a bacterial virus, Biochim et biophys. acta 9, 419-429)

in animal viruses are treated in other chapters

Ionizing and ultraviolet radiation have provided important tools in the analysis of virus multiplication Chemical inferences may be drawn from results obtained in this way as discussed in part in later portions of this chapter; however, such data and conclusions are discussed particularly in Chapter 7

COMBINED BIOLOGIC AND MORPHOLOGIC DATA

The advantages of combining the attacks of several disciplines in analyzing virus-infected cells may be recorded in the following examples: Phage-infected bacteria were disrupted by decompression shock, and the exposed internal macromolecular structures of the cell were examined in the electron microscope (Levinthal and Fisher, 1952). As can be seen in Figure 25A, intact virus particles do not begin to appear within the cell until the second half of the latent period The first sign of the formation of new virus is the appearance of the hollow shells of the heads of virus particles These structures have been called "doughnuts" The doughnuts have been shown to contain phage antigens unlike those involved as adsorption sites (Lanni and Lanni, 1953) The relation of the formation of doughnuts to intact phage is given in Figure 25B

In an extension of this study, the contents of infected cells inhibited by proflavin were studied (DeMars et al, 1953) This compound allows lysis of infected bacteria to occur without liberation of infectious phage (Foster, 1948). When such inhibited cells are disrupted at various times after infection, it is found that the doughnuts appear in the bacteria at the usual time but are not replaced by mature virus It was concluded that proflavin affects terminal organizing steps in the completion of phage particles

Although the conditions for such an integrated kinetic study of animal virus multipli-

disappears as virus appears, suggesting that later the new RNA may be incorporated into virus. As pointed out earlier, uracil, unlike the other bases, is limiting in determining the amount of virus synthesized in this system

Very little has been done on the course of virus multiplication in other plant systems. An important limitation on the development of information comparable with the above is the fact that the yields of many other plant viruses may be as low as 0.1 to 0.01 per cent of that of tobacco mosaic virus.

In the multiplication of a recently studied crystallizable insect virus, isolable virus can attain a weight approximately 25 per cent of that of its host. This crystallizable virus contains DNA but multiplies in the cytoplasm of the fat-body cells of the larvae of *Tripula paludosa*, the crane fly (Williams and Smith, 1957). This situation evidently presents an interesting opportunity to the biochemist.

However, the multiplication of most animal viruses does not appear to attain such favorable weight ratios. In most instances of viral infection of human tissues virus production appears to represent only a very small proportion of that observed in phage TMV or insect virus infections. For these reasons, the methods of mass analysis indicated above are not generally practicable, since in most cases only a very small proportion of an infected cell can be expected to represent virus-specific products. However, the application of isotopic technics has markedly enlarged the scope of chemical analysis as applied to this problem.

Isotope Technics. In this section will be considered a number of types of experiments which both increase the sensitivity and the specificity of chemical measurement and have assisted in the clarification of the mechanism of virus multiplication.

Some components of interest are synthesized in small amounts, either because their synthesis may have just begun in the course of an experiment, or their role does not require large amounts or their accumulation is not permitted as a result of turnover in virus-infected cells. In some instances there are problems of isolation and estimation which make it difficult to obtain quantitative data. In all of these cases the use of highly radioactive isotopes has proved to be very useful in the following ways.

1. In the analysis of a radioactive component present in small amounts, known amounts of the component can be added in a nonradioactive form to the mixture from which the component is later to be isolated. The radioactivity of the isolated material, corrected for dilution by the added nonradioactive material, will permit the calculation of the initial concentration of the radioactive component [illegible]

1957). This application of the method is the converse of the usual use of the method, as in the amino acid analysis of a protein in which an isotopic amino acid may be added to a protein hydrolysate, where the isotopic compound is diluted by the unlabeled amino acid. In the latter case the dilution of radioactivity by the amino acid whose concentration is to be determined provides an estimate of its initial concentration in the protein hydrolysate.

2. When P^{32} is incorporated into T_2-infected bacteria, it is found that a small amount (1 to 3% of the P of the fraction) is incorporated into the RNA fraction and behaves like RNA. Such an amount could not be detected without the use of isotopes. However, it is difficult to prove that such a small portion of the RNA fraction is indeed RNA and is not an impurity. Degradation of the RNA and fractionation revealed the presence of P^{32}-containing ribose nucleotides which could have been derived only from RNA (Volkin and Astrachan 1957). In this fractionation, the isotopic nucleotides were diluted by the preformed nonradioactive ribose nucleotides of the uninfected cell.

In this experiment, the isotope contents of the 4 ribose nucleotides were found to exist in ratios characteristic of the ratios of the deoxynucleotides of virus DNA and very dissimilar from the ratios of the nucleotides in host RNA, as can be seen in Table 4. Furthermore, it was found that this new synthesis of RNA occurs in a part of the cell readily separable from most of the cell RNA. Such data suggest the following possibilities.

1. Newly synthesized ribose nucleotides may be precursors of deoxyribonucleotides, even as has been suggested in the formation of deoxyribonucleotides in normal bacteria on the basis of isotopic evidence (Lanning and Cohen 1955).

2. The newly synthesized polymeric RNA of the infected cell may play a functional role in the specific synthesis of phage polymers.

the tryptophan analogue inhibits the consequent production of viral DNA In the former instance, infection with damaged virus particles produces unusual metabolic patterns For example, T_2-particles can be prepared which have been burst osmotically to release their DNA and consist essentially of the adsorbable outer shell of the virus (Anderson, 1949; Herriott, 1951) On infection with such phage ghosts, the bacteria stop multiplying, do not produce virus and are severely inhibited in protein synthesis for a considerable period (French and Siminovitch, 1955)

On the other hand, infection of bacteria with ultraviolet irradiated virus inhibits the synthesis of DNA (Cohen, 1948, Cohen and Arbogast, 1950) but does not inhibit initial protein synthesis obtained in normal infection (see Fig 26A) In addition, low molecular precursors of viral DNA accumulate within the infected cell (Cohen, 1951) If the level of ultraviolet irradiation does not prevent multiplicity reactivation in infected cells (Chap 7), it can be shown that viral DNA synthesis is an all-or-none phenomenon requiring a full complement of active viral genetic units (Cohen and Arbogast, 1950).

In analyzing the changes in DNA in T_2-infected cells by chromatographic methods, it has been shown that DNA containing cytosine, i e, host DNA, disappears, and DNA containing viral-specific 5-hydroxymethyl cytosine appears (Hershey et al, 1953; Vidaver and Kozloff, 1957) The ratios of the increments in the purine and pyrimidine bases in infected cells are essentially those observed in virus DNA (Barner and Cohen, 1954); these ratios are different from the ratios of the bases in bacterial DNA In the latter study it was shown that when T_2 infects a thymine-requiring bacterium, the cell is induced to synthesize two pyrimidines—thymine as well as hydroxymethyl cytosine, which the normal bacterium was previously incapable of synthesizing.

In the various experiments relating to T_2-infected cells described above, it was found, as shown in Figure 26A, that the synthesis of various components, including viral specific components such as DNA, occurs at a constant maximal rate. Indeed, the rates at which infected cells incorporate essential elements—phosphorus, nitrogen and carbon—approach the rates of incorporation into normal growing cells. These syntheses are then independent of the number of virus particles present within the infected cell This would suggest that the syntheses are not effected by the tiny infecting virus particle but are rather a function of the relatively invariant enzymatic equipment of the host cell These enzymes continue to make most of the normal building blocks which, instead of entering nucleic acids and proteins, are diverted to the formation of the viral polymers

In some other phage systems, such as T_3 and T_7, the DNA of the bacterial nucleus is entirely degraded and resynthesized into viral DNA without a de novo synthesis of additional DNA, thus, no significant alterations of DNA content can be detected in infected cells (Labaw, 1951, Putnam et al, 1952; Kozloff, 1953)

In some other systems, e g, the T_6-*E coli* system or induced lysogenic systems, other patterns of polymer production may be observed In Figure 26B are presented data on the synthesis of the nucleic acids in lysogenic *B megatherium* after induction with ultraviolet light. The induction results in the mass production of virus and lysis It can be seen that after an initial lag, the production of DNA occurs at a stimulated rate However, unlike the T_2-*E coli* case, there is a marked continuing net synthesis of RNA until the moment of lysis

The amounts of certain plant viruses such as tobacco mosaic virus (TMV) in infected plants can represent very considerable proportions of the total cell protein, TMV may equal as much as 10 per cent of the solids of diseased leaves In these instances a study of the distribution of various protein and nucleic acid fractions may be expected to throw some light on events during multiplication A series of cyclic changes has been observed in soluble protein, insoluble protein, and RNA in infected and uninfected cells, and differences between the contents of these fractions have been related to virus synthesis (Basler and Commoner, 1956). Thus, virus biosynthesis is associated with a net increase in plant proteins (Commoner et al, 1953) and seems to be most closely associated with a buffer-insoluble cell component. After infection there is a synthesis of new RNA, and this material

conditions For example, cells labeled in cytosine with C^{14} and infected in unlabeled media could be observed to lose DNA-cytosine-C^{14} and accumulate HMC-C^{14} (Hershey et al, 1954) In other experiments it was also shown that bacterial RNA contributed to the formation of virus DNA Thus, if cells grown in the presence of C^{14}-glucose are permitted to grow briefly in the absence of isotope to exhaust intermediary metabolites, and then infected in C^{12}-glucose, it is found that the total amount of radioactivity in intracellular DNA-bases increases, whereas the label in RNA decreases slightly (10 to 20%) but significantly.

A modification of this type of experiment, i e, shifting from labeled to unlabeled metabolites, has been used extensively to map metabolic pathways The technic attempts to assess the effects of simultaneously offering two possible sources of metabolites, only one of which is labeled If the labeled compound is indeed the precursor, the newly made substance will contain its radioactive atoms If the unlabeled component offered will enter the metabolic path to provide unlabeled atoms for the synthesis of the component investigated, such an unlabeled source will compete as a source of atoms and will reduce the number of radioactive atoms incorporated per unit synthesized Such a technic is known as "isotope competition" (Roberts et al, 1955) A typical experiment is given in Table 5

It can be seen that fed adenosine dilutes isotope in the formation of bacterial adenine and guanine but only slightly of bacterial ribose On the other hand, C^{12}-pyrimidine nucleosides do not seriously affect isotope incorporation from C^{14}-glucose into purines but do provide competitive unlabeled ribose for RNA synthesis

Many comparable competition experiments have been done with normal and virus-infected bacteria (Siminovitch and Graham, 1955, Hershey et al, 1954). For example, it has been shown that if C^{14}-uracil is fed to a uracil-requiring strain of *E coli*, the organism contains the C^{14}-pyrimidine in its nucleic acid pyrimidines Simultaneously offered C^{12}-cytosine deoxyriboside will dilute the isotope content of newly synthesized thymine However, no hydroxymethylpyrimidine offered at the free base or nucleoside level, including hydroxymethylcytosine deoxyriboside, will compete with C^{14}-uracil in entering infected cells to become T_2 or T_6 pyrimidines (Cohen et al, 1957) These compounds do penetrate infected cells and therefore are excluded as possible intermediates in the biosynthesis of viral pyrimidines

Such an experiment does not exclude pyrimidine nucleotides as intermediates However it is not possible to feed such a nucleotide in hopes of its entrance into a normal metabolic pathway, since experiments with P^{32}-labeled nucleotides have indicated the release of the P^{32} and its redistribution to other nucleotides (Lesley and Graham, 1956). In this case, one must seek a new method of increasing permeability to such compounds or study nucleotide metabolism in cell-free extracts

As is considered below, a number of investigators have undertaken to label the infecting virus particles and to determine the fate of parental viral substance In this way, it was found that some of the phosphorus atoms of the parental nucleic acid of a T-even phage particle appeared in the progeny It can be asked if this transferred P derives from intact viral nucleic acid or represents the re-utilization of P from degraded nucleic acid Hershey et al (1954) performed competition experiments to approach this problem These workers prepared uniformly labeled C^{14}-glucose The isotopic phage was used to infect unlabeled bacteria in unlabeled medium and the DNA bases of the progeny isolated from

TABLE 5 UTILIZATION OF C^{14}-GLUCOSE FOR NUCLEIC ACID SYNTHESIS IN *E coli* (Bolton, 1954)

		Specific Radioactivity (counts/sec/micromole)			
		Bacterial Adenosine		Bacterial Guanosine	
Culture	C^{12} Supplement	Adenine	Ribose	Guanine	Ribose
1	None (control)	20	16	20	14
2	Adenosine	2	15	5	11
3	Cytidine	17	7	13	5 5
4	Uridine	20	8 5	20	4

TABLE 4 RATIOS OF INCORPORATION OF P^{32} IN RNA OF T_2-INFECTED *Escherichia coli* CONTRASTED WITH HOST AND VIRAL NUCLEIC ACIDS (Volkin and Ostrachan, 1957)

NUCLEOTIDES	PER CENT DISTRIBUTION OF BASES IN *E coli* RNA	PER CENT DISTRIBUTION OF P^{32} IN T_2-*E coli* RNA	PER CENT DISTRIBUTION OF BASES IN T_2 DNA
Cytosine *	23	18	17
Guanine	31	22	18
Uracil *	23	30	32 5
Adenine	23	30	32 5

* Virus DNA contains thymine, instead of uracil, and cytosine is replaced by 5-hydroxymethyl cytosine.

Although neither hypothesis is rigorously proved, it is possible that both are correct. When P^{32} ... for ... radi... P^{32} ... DNA continues to incorporate P^{32} while the content of this isotope in RNA falls reciprocally Thus, the P^{32} ribose nucleotides appear to be precursors of DNA deoxyribotides at some level of organic phosphorus compounds It is not known whether these levels of precursor organization are nucleotides or polynucleotides The process of making isotope available to infected cells for brief intervals is termed a "pulse" experiment and will be referred to again below

Precursor relations of virus components have been examined by 3 main types of isotope experiments. In these, a labeled compound may be present (1) in the medium at various ... in the ... (3) in ... multipl... involves continuity of viral substance These experiments were first developed in phage systems and have since begun to be extended to ...

In ... source ... a T-e... eration ... labeled cells, i e, cells grown in P^{32}-containing media but infected in unlabeled media or conversely infected in unlabeled cells in a medium containing P^{32}. Virus was isolated, and the radioactivity of the P was determined as compared with the P of labeled host or labeled medium, respectively. It was found that from 70 to 80 per cent of the P of the virus was derived from the exogenous P of the medium

By means of a selective labeling of the host nucleic acids with nucleic acid components, i e, with C^{14}-adenine and thymine (Kozloff, 1953), it was shown that the main source of the host contribution, i e., 20 to 30 per cent of the T_6r^+ nucleic acid, was host DNA By infecting cells, previously labeled in their pyrimidines through growth in C^{14}-orotic acid, and liberating and isolating virus at various stages ... This was taken to mean that components of the host nucleic acid do not fulfill a role as genetic determinants within the T-even phages, a fact substantiated by the subsequent discovery that phage nucleic acid is devoid of cytosine, a base found in host DNA (Wyatt and Cohen, 1953)

As summarized by Kozloff (1953), it has been shown in studies of the T-odd phages by these technics that from 60 to 90 per cent of the viral DNA-P and DNA-N came from the host cells, which show little or no synthesis of DNA after infection

In an extension of these experiments, Stent and Maaloe (1953) and Labaw (1953) introduced the method of rapidly adding or diluting isotope at intermediate stages of the multiplication cycle, i e, the "pulse" experiment, and observing the effect on the radioactivity of the liberated infectious phage By this technic the former workers showed that the average time spent by phosphorus atoms between assimilation and incorporation into the intact phage was about 14 minutes. In this process, however, newly assimilated P mixes at some metabolic level with P derived from host DNA

In further refinements it was realized that intermediate products of the infected cells rather than completed liberated phage could also be determined under various labeling

conditions. For example, cells labeled in cytosine with C^{14} and infected in unlabeled media could be observed to lose DNA-cytosine-C^{14} and accumulate HMC-C^{14} (Hershey et al, 1954). In other experiments it was also shown that bacterial RNA contributed to the formation of virus DNA. Thus, if cells grown in the presence of C^{14}-glucose are permitted to grow briefly in the absence of isotope to exhaust intermediary metabolites, and then infected in C^{12}-glucose, it is found that the total amount of radioactivity in intracellular DNA-bases increases, whereas the label in RNA decreases slightly (10 to 20%) but significantly

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resulting lysates were analyzed for radioactivity In parallel experiments the unlabeled compounds—adenine, guanine and thymidine—were added in an attempt to compete with the labeled bases in the infecting phage DNA. No change in the distribution of isotope was obtained in any of these competition experiments as contrasted with the control. It would appear that parental DNA is not broken down to a level at which the competition of exogenous derivatives could occur, and it was concluded that DNA is transferred in large pieces which might conceivably be genetically functional.

Isotopic technics enable the investigator to study the fate of the major components of a system of infected cells, i e , virus, cell, or medium. Although the examples given above have been derived mainly from questions on the role, the origin and the fate of nucleic acids in phage systems, these kinds of experiments can also be applied to other cell systems and to problems involving types of other metabolism, e g , protein In the study of protein metabolism, an isotope of sulfur, S^{35}, an element not found in nucleic acid, has been particularly useful, as have C^{14}-amino acids and the stable isotope, N^{15} P^{32} has not yet been of interest in the study of protein metabolism in infected cells in the absence of knowledge of protein-bound P in viruses or of its role in many metabolic events

In animal virus systems it has been difficult until recently to perform clearcut isotope experiments Many experiments have been done since 1949 on P or C isotope uptake in intact infected animals or in infected eggs. The early history of such experiments using influenza virus, vaccinia virus, Theiler's virus, etc., has been summarized by Blank (1956) In such experiments it has been customary to note a slight increase in isotope uptake in animal, egg, or one or another fraction on infection as contrasted with control Occasionally, the incorporation into virus has been studied as well. For the most part, however, the biologic systems employed have not been used to permit single cycles of multiplication and have precluded extensive incorporation into virus or into components which might be purified sufficiently to warrant designation as viral components.

However, isotope experiments have begun

the effect of poliomyelitis infection on P^{32} utilization in the HeLa cell and have observed an accelerated uptake into nucleic acid and phospholipid fractions of such cultures

In the earliest experiments with minced one-day-old mouse brain infected with Theilers GVII virus, P^{32} uptake was stimulated in the RNA fraction but not in DNA (Rafelson et al , 1949). Such a result may be taken to suggest that this virus is probably an RNA virus, as is human poliomyelitis virus. Subsequently, it was determined that P^{32} incorporation into the nucleic acid of the smallest particulate fraction of mouse brain was stimulated maximally (Moldave, 1954). Thus, such experiments as unsatisfactory as they are, nevertheless permit a beginning in the orientation of biochemical thinking concerning a virus difficult to isolate and purify When labeled virus has been isolated, as in studies with influenza virus, it has been used to study viral adsorption (Liu, 1956), viral composition (Graham, 1950, Hoyle et al , 1954, Franklin et al., 1957) and viral penetration (Hoyle and Frisch-Niggemeyer, 1955, Hoyle and Finter, 1957)

Most isotopic studies of plant virus multiplication, i e , the biosynthesis of tobacco mosaic virus, have used the stable isotope, N^{15}, to analyze the interrelations of normal tobacco protein, the amino acid pool and virus (Meneghini and Delwiche, 1951, Commoner et al , 1953)

The Synthesis of Incomplete Influenza Virus. It was shown first in the multiplication of turnip yellow mosaic virus that infected plants elaborated both an infectious spherical nucleoprotein and a noninfectious spherical protein shell which appeared to be identical with the virus except for the lack of the internal core of virus RNA (Chap. 2). Studies of Commoner et al. (1953) suggest that the lack of availability of RNA components such as uracil may indeed limit tobacco mosaic virus production It has been supposed that virus infection may lead to the independent synthesis of RNA and protein units which eventually assemble to form the complete nucleoprotein or in the absence of sufficient nucleic acid organize protein particles, i e , incomplete virus.

Under certain conditions of infection of chick embryos with influenza virus, the yields contain a high proportion of noninfectious hemagglutinins in addition to infectious virus particles On an additional cycle of infection

with such a harvest, the progeny are almost entirely devoid of infectivity. Such noninfectious hemagglutinating particles have been termed "incomplete," and indeed the study of Ada and Perry (1955) has revealed that the RNA content of a population of purified virus from infected allantoic fluids is proportional to the logarithm of the ratio of infectious to hemagglutinating particles. Thus, noninfectious particles lack a complete complement of RNA, are structurally less firm in electron micrography, and indeed appear to have a lower sedimentation coefficient than do preparations of highly infectious virus. In confirmation of these results, Henle (1956) has described experiments on P^{32} uptake in the production of incomplete influenza virus. He reports that compared with fully infectious influenza virus such isolated "incomplete" particles contain a lower percentage of the total P^{32} in the nucleic acid and perhaps a relatively higher percentage in the lipid fraction. The results to date are consistent with the view that influenza virus consists of small hemagglutinating particles plus a nucleoprotein component enclosed in a lipid envelope, that, as suggested by Hoyle (1952), the nucleoprotein element is of particular significance in initiating multiplication, and that in the formation of incomplete virus there is just not enough complete nucleoprotein to go around. In addition, the point may be made that the hemagglutinin must be on the surface of the virus particle. The structural and isotopic work on fowl plague virus, which is closely related in morphology, composition and multiplication to influenza virus, also appears to substantiate this general picture (Wecker and Schafer, 1956, Schafer, 1957). It is evident that structural and isotopic studies are beginning to clarify the multiplication of influenza virus, however, the point may be made that these studies must also be set within a kinetic analysis of the sequence and the mode of formation of all the various virus-specific units.

Energy Requirements for Virus Multiplication

It had been postulated some years ago that the intracellular multiplication of viruses might resemble an autocatalytic conversion:

1. Trypsinogen $\xrightarrow{\text{trypsin}}$ trypsin
2. Virus precursor $\xrightarrow{\text{virus}}$ virus

in which the virus precursor would be a large organized preformed nucleoprotein. The data presented above already indicate that this model does not typify the multiplication of any known virus. The net synthesis of the complex substances from simple nutrients indicated a far more complex array of metabolic steps. Such syntheses evidently have energetic requirements of a far different quality and magnitude than that represented by the hydrolytic conversion of the precursor trypsinogen to trypsin. The synthesis of virus polymers requires a considerable expenditure of energy, at least in raising intermediary metabolites to the level of producing the activated amino acids and nucleotides which can participate in protein and nucleic acid synthesis. Such activated compounds contain high energy bonds which embody a sufficiently high group transfer potential to permit the addition of activated amino acids to amino acids to extend peptide chains and the addition of activated nucleotides to extend polynucleotide chains as well as to participate in a multitude of reactions involving the activation of other intermediary metabolites.

It is evident then that virus multiplication must utilize the major sources of energy metabolism available to normal cells. In anaerobic and aerobic cells this involves the development of oxidative phosphorylation via hydrogen transport. Other mechanisms are also available whereby oxidation-reduction is tied more directly to the development of high energy bonds. Thus, in anaerobic glycolysis the conversion of 1 mole of glucose to 2 moles of lactic acid occurs with the net production of 2 moles of adenosine triphosphate (ATP). In the oxidation of pyruvate, the free energy evolved in the conversion of a 2-carbon fragment at the oxidation level of acetaldehyde to that of acetate may be conserved in the formation of a thioester of acetate, which can then be transferred or exchanged to form other high energy bonds. Since enzymes controlling oxidation-reduction are apparently not present in the viruses, we are concerned in the main then with host systems of metabolism. It would not be anticipated that these systems would be seriously affected during active

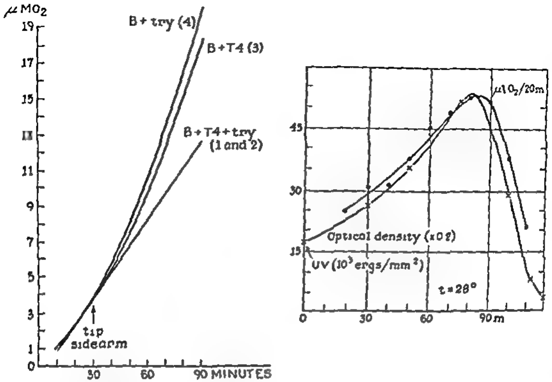


FIG 27 (A, *Left*) The effect of T4r+ virus adsorption on the rate of O_2 consumption of culture of *E coli* strain B in lactate medium Try-tryptophan, the adsorption cofactor for T4 (Cohen, S. S., and Anderson, T. F, 1946, Chemical studies on host-virus interaction. I The effect of bacteriophage adsorption on the multiplication of its host, *Escherichia coli* B J. Exper Med *84*, 511-523) (B, *Right*) Measurement of rate of respiration after induction of *B. megatherium 899* by ultraviolet light (Siminovitch, L., 1953, Biochemical modifications of the bacterial host during bacteriophage development, Ann Inst Pasteur *84*, 265-273)

polymer and virus synthesis, although the diversion of metabolic products from maintenance and repair mechanisms may eventually result in cell damage Conversely, it can be expected that the inhibition of major routes of energy metabolism will seriously affect the course of virus production. In general, these expectations are realized in most systems examined, although some few exceptions have been reported

It was first observed with *E coli* infected with T_2 or T_4 phages that infected cells maintained the rate of O_2 consumption or respiratory quotient obtained with growing cells immediately prior to infection (Cohen and Anderson, 1946). However, although uninfected cells exhibited the normal increase of respiration as a result of growth and division and the biosynthesis of respiratory systems, T_2 or T_4 infected cells did not increase their O_2 consumption, since normal growth and the synthesis of respiratory enzymes ceased. These data are represented in Figure 27A

Similar results have been obtained with a number of other virulent phages infecting *Pseudomonas aeruginosa* and T_5 infecting *E coli* (Holliman et al, 1952) On the other hand, following induction of lysogenic *B megatherium*, in which growth and RNA synthesis continue until lysis, the rate of O_2 consumption increases as in Figure 27B (Siminovitch, 1953)

Phages are known for anaerobic organisms, and a number of investigators have compared aerobic and anaerobic virus production in facultative organisms Fowler (1950) has studied this for T_2 infecting *E coli* and has shown that *E coli*, adapted to anerobic growth on glucose, is capable of producing normal amounts of virus Since it is well known that

high-energy compounds can be developed during anaerobic growth, it should not be surprising that polymer and virus synthesis may continue under these conditions

However, if the bacteria had not been adapted to anaerobic conditions prior to infection, virus production did not occur Infection by T_2 of aerobic *E coli* in the absence of O_2 resulted in lysis without virus production (Cohen, 1949). As summarized by Adams (1954), this type of lysis is related to the phage used and the medium in which the organism is grown The effect may be expected to be maximal in a lactate medium where it was first observed, in view of the fact that *E. coli* infected with T_2 or T_7 accumulate pyruvate, indicating a decreased ability to handle this substrate on infection (Spizizen, 1957b) In the T_2-*E coli* system the elimination of an energy source and the provocation of lysis can be effected with inhibitors such as cyanide (Cohen, 1949) and 2,4-dinitrophenol (Heagy, 1950) The penetration of phage DNA requires the puncturing of the cell wall, resulting in an increased leakage of metabolites from infected cells, which is repaired in later stages of the multiplication cycle (Puck and Lee, 1954, 1955) The lysis observed to occur on the elimination of an energy supply or resulting from a high multiplicity of infection (lysis from without) has been suggested to arise from the failure of the repair mechanism to operate in infected cells, a mechanism which may be presumed to involve extensive protein synthesis

Many studies have been made comparing respiration in healthy and virus-infected plants In one of the most careful comparisons of healthy and mosaic-diseased tobacco plants from the time of inoculation, it was concluded that the respiration rates are essentially similar until the disease became systemic (Gladstone, 1942). Numerous reports have appeared of increased and decreased respiration rates in infected plants, in many instances increased respirations have been interpreted as reflecting translocations of utilizable substrates (Wynd, 1943, Bawden, 1950)

In animal virus systems most investigators have failed to observe inhibition of respiration of a host tissue during propagation of virus Such results have been obtained for the polyhedral disease of silkworms (Gratia et al, 1945), and infections with viruses of murine encephalomyelitis (Pearson and Winzler, 1949) Newcastle disease and Western equine encephalitis (McLimans et al, 1950), influenza, Ackermann, 1951, Tamm, 1956), feline pneumonitis (Moulder and Weiss, 1951) and vaccinia virus (Overman and Tamm, 1957) Of course, after cytopathology develops, a decrease in respiratory rate is common, as in herpetic infection in chick liver and heart, which results in mitochondrial destruction (Ackermann and Kurtz, 1952), or late in vaccinial multiplication in chorio-allantoic membranes (Overman and Tamm, 1957)

In a number of infections, e g, feline pneumonitis (Moulder and Weiss, 1951), murine encephalomyelitis (Pearson and Winzler, 1949), the anaerobic glycolysis of infected tissue is stated to be unchanged However, in a variety of instances, this function is reported to be altered In the studies of Smith and Kun (1954) extracts with infections of myxoma, fibroma, herpes simplex, rous sarcoma, swine influenza, Newcastle disease and rabies, the rates of production and O_2 consumption of the chorio-allantois were unchanged as contrasted with the normal tissue while the rate at which extracts of infected membranes produced acid from hexose diphosphate (a measure of anaerobic glycolysis) was increased markedly Sterile inflammation of the membrane did not produce this result. A stimulatory effect on anaerobic and aerobic glycolysis by poliovirus infection of monkey kidney cells in tissue culture has also been observed (Levy and Baron, 1957) As will be noted below, evidence has been obtained for an increase in the use of a pathway of anaerobic glycolysis in T_2-infected bacteria

Although many workers have reported a diminution of virus production in anaerobic conditions, it has been observed recently that poliovirus will reproduce similarly in the HeLa cell in anaerobic as well as aerobic conditions (Gifford and Syverton, 1957) The HeLa cell has a normally high rate of aerobic glycolysis and may be thought of as having a most active functioning anaerobic Embden-Meyerhof pathway even in aerobic conditions. Thus, it may be adapted to anaerobiosis before infection, unlike some other systems studied

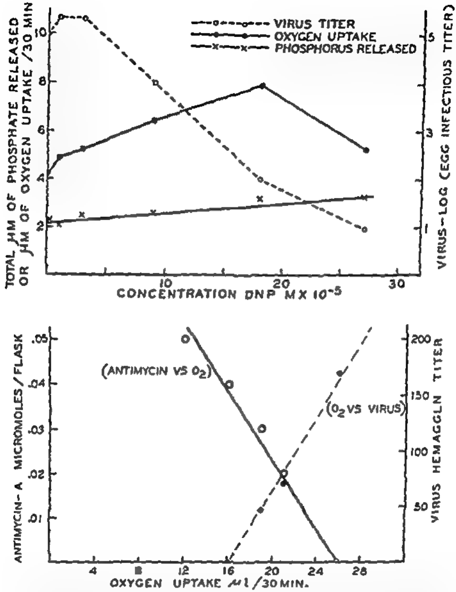


FIG. 28. (A, *Top*) Uncoupling effect of dinitrophenol (DNP) on propagation of influenza virus in the chorio-allantoic membrane of the chick embryo (Ackermann, W W, and Johnson, R. H., 1953, Some energy rela- [illegible]

Virus and Rickettsial Infections, pp 101-110, New York, Blakiston)

It may be noted that many workers have reported phenomena suggestive of an increase in the permeability of infected cells in a manner analogous to that described for phage systems In the absence of a rapid repair mechanism, anaerobiosis may reduce energy supply and merely augment this effect, as it does in phage systems The inhibition of anaerobic glycolysis by leukocytes as a result of treatment with the influenza viruses or by the receptor-destroying enzyme of *Vibrio cholerae* (Fisher and Ginsberg, 1956) has been interpreted to suggest that a permeability increase with loss of essential phosphorylated intermediates may have been responsible for the observed inhibition It is noteworthy that such abused leukocytes, when supplied with phosphorylated intermediates, such as fructose-6-phosphate, were able to glycolyse and phagocytize, in contrast with their loss of these functions with glucose as exogenous substrate.

Inhibitors have been useful in demonstrating energetic requirements for virus multiplication, and particularly the contribution to energy requirements which may stem from oxidative systems As given in Figure 28A, uncoupling of oxidative phosphorylation from respiration by dinitrophenol reduces influenza virus yield in chorio-allantois in proportion to the increase in O_2 consumption which this agent stimulates (Ackermann and Johnson, 1953, Eaton, 1952) Dinitrophenol also stimulates the ATPase of the membrane, resulting in the formation of inorganic phosphate in the medium Sodium malonate (Ackermann, 1951) and sodium fluoroacetate (Mogabgab and Horsfall, 1952), which also inhibit influenza virus production, prevent the normal functioning of the tricarboxylic acid cycle, which in oxidizing acetate both facilitates oxidative phosphorylation and supplies important intermediates for protein synthesis Other inhibitory agents, such as antimycin A, which interferes with electron transport (Ackermann, 1951), also prevent influenza virus production, as in Figure 28B Similar agents include arsenite, cyanide, azide, etc, which inhibit the multiplication of phage (Dolby, 1955), vaccinia virus (Thompson, 1947), and feline pneumonitis virus (Moulder et al, 1953)

It must be emphasized that although the use of the inhibitors and the exploration of these peripheral areas of energy metabolism have established the gross generalities stated at the beginning of this section, the core of the biochemical problem cannot be approached by these primitive technics This central problem may be stated to be that of the nature of the energetic coupling involved in the synthesis of the viral polymers It is not known whether these synthetic reactions differ in any significant respect from the synthesis of comparable host polymers, whose mechanisms of synthesis are also still unknown

Enzyme Changes in Virus-Infected Cells

Direct and indirect approaches have been employed in attempting to seek or assess changes in the enzymatic activities of virus-infected cells It is possible to disrupt cells and assay directly for enzyme content As noted earlier, to be interpretable any observed changes in enzyme content require a knowledge of both the stage of infection analyzed and the percentage of infected cells among the cells studied A second type of analysis has explored the rate at which infected cells use given substrates, incorporate such substrates in fractions of infected cells or accumulate or excrete various metabolic products or intermediates A number of instances have already been recorded of this type of study, as in the analysis of respiration or glycolysis, or the incorporation of P^{32} in nucleic acids, etc It is immediately evident that the extrapolation from an observation of substrate utilization by intact cells to a conclusion of a change in enzyme content or activity must be approached with caution, since a change in the rate of utilization may arise from permeability changes, a shift from one biosynthetic pathway to another, structural alteration, etc

To exemplify this point, we may refer to the fact of pyruvate accumulation in virus-infected bacteria (Spizizen, 1957) The additional observation was made that various compounds, probably including cocarboxylase, which are capable of stimulating pyruvate utilization, leak from such infected cells As indicated above, such a permeability change could account for the inhibition of glycolysis by leukocytes treated with influenza virus

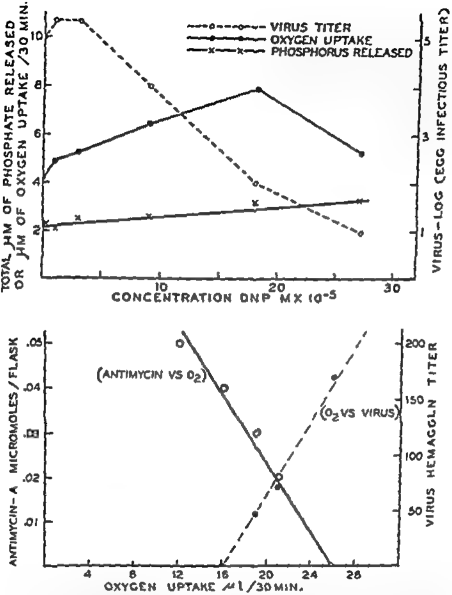


FIG 28. (A, *Top*) Uncoupling effect of dinitrophenol (DNP) on propagation of influenza virus in the chorio-allantoic membrane of the chick embryo (Ackermann, W W, and Johnson, R. B., 1953, Some energy relations in a host-virus system, J. Exper Med *97*, 315-322) (B, *Bottom*) Relationships of antimycin-A concentration on oxygen uptake and influenza virus propagation in chorio-allantoic membrane (Ackermann, W. W.

drogenases of glucose-6-phosphate and 6-phosphogluconate, were present in amounts equivalent to those of the normal cells, i e, an amount capable of handling 40 per cent of the total glucose utilized (Scott and Cohen, 1953; Scott, 1956). Another type of test made use of the physiologic irreversibility of the formation of phosphogluconate. When *E. coli* is adapted to gluconate, the bacteria form a gluconokinase which converts gluconate to 6-phosphogluconate, as in the diagram on page 78 (Cohen, 1951). When such adapted cells are infected by T_2, in the presence of gluconate-1-C^{14}, essentially all of the isotope appears in CO_2 as a result of the oxidative decarboxylation of 6-phosphogluconate. Nevertheless, the infected cell elaborates virus and virus DNA (Cohen and Roth, 1953) Thus, at least all but one of the enzymes of the phosphogluconate pathway are not inhibited and are capable of functioning in infected cells, if compelled to by the appropriate choice of substrate; however, these enzymes tend not to be used if glucose is offered The glucose-6-phosphate dehydrogenase, not tested by this method, had been shown to be present by direct assay, and by earlier isotope analyses It was concluded that virus infection appears to inhibit the use of the phosphogluconate pathway by controlling other levels of metabolism

Thus, although O_2 consumption during utilization of glucose showed no apparent change, this constancy masked a shift in balance between alternative paths of carbohydrate metabolism This technic has not yet been extended to other viral systems, although now being applied widely in the analysis of carbohydrate metabolism. Recently, an increased use of the phosphogluconate pathway has been reported for rust-infected wheat (Shaw and Samborski, 1957)

Other functions in such infected bacteria show more evident changes in the utilization of given substrates Thus, certain nucleic acid derivatives such as adenine, adenosine, and thymidine disappeared at greater rates in infected cultures which, nevertheless, deaminated cytosine derivatives or cleaved nucleosides more slowly (Friedman and Gots, 1953) On the other hand, a number of different enzymes in extracts of infected cells, e g, apyrase, ribonuclease, etc, are said to be present to a similar degree in infected and normal cells (Pardee and Kunkee, 1952)

A very interesting change in the deoxyribonuclease content of infected *E. coli* has been observed (Pardee and Williams, 1953; Kozloff, 1953) This is an increase in the enzyme which has been correlated with the destruction of a small fraction of DNAase-inhibitory RNA However, although this increase has been described earlier as an activation, it may be a de novo formation of enzyme, since the increase is specific for T-even phages, and occurs only when phage DNA is injected and an energy source is supplied (Kunkee and Pardee, 1956).

It was shown very early that bacteria infected by certain virulent phages could not be induced to use a new carbon source (Monod and Wollman, 1947), and it was assumed that this exclusion of induced biosynthesis was a reflection of the priority for the synthesis of phage polymers which infection imposed on the cell This priority system broke down in certain lysogenic systems (Jacob, 1953) which could adapt to new substrates after induction Evidence is beginning to accumulate to suggest that the exclusion of enzyme synthesis may not hold entirely in virulent systems either Indeed, it now appears that the inhibition of synthesis of protein or of inducible enzymes in T_2 systems is an early effect of infection, unrelated to the phage polymer biosynthesis directed by injected DNA, since such an inhibition can be produced by attachment of T_2 ghosts which arrest bacterial multiplication and biosynthesis (French and Siminovitch, 1955)

In the phage infection of certain strains of *Klebsiella pneumoniae*, an enzyme is elaborated which is partially attached to the virus and hydrolyzes the capsular polysaccharide of the bacterium (Park, 1956) Comparable enzymes had been found earlier in other phage-bacterial systems, as summarized by Adams and Park (1956), and could not be detected in uninfected cells or culture media In these instances, it was not shown whether infection merely uncovered an inhibition; indeed, the new enzyme might have arisen as a result of the liberation of inducing products in the initial penetration of the capsule

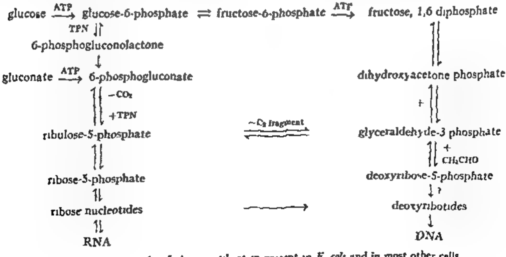
In the case of the formation of 5-hydroxymethyl deoxycytidylic acid in *E. coli* in-

(Fisher and Ginsberg, 1956). A variety of changes in substrate utilization by chorioallantois infected with influenza virus have been recorded. Pyruvate, a difficulty penetrable substrate, is taken up at a slightly greater rate than in normal tissue (Wielgosz, 1957), as are N^{15}-alanine, C^{14}-glycine, or P^{32}-phosphate (Huttin et al, 1954, van den Ende, 1950) The latter results were shown to be due mainly, if not entirely, to increased permeability in the membrane, and these effects of infection could be simulated by treatment of normal tissue with hyaluronidase

A wide variety of metabolic changes has been observed in phage-infected cells in which analyses at the enzymatic level have also been attempted. Of these studies of infected bacteria, one of the most interesting cases began with the demonstration of no apparent metabolic change at all. It has been noted that the rate of O_2 consumption and respiratory quotient does not change after infection with a T-even phage However, the pattern of synthesis, particularly with respect to nucleic acid, changed markedly after infection. It was shown that in *E. coli*, ribose arose predominantly from the oxidative phosphogluconate pathway (Lanning and Cohen, 1954), one of two alternative pathways of glucose utilization, of which the other is the anaerobic Embden-Meyerhof pathway A simplified relation of these alternative routes is given in the accompanying diagrams; these routes and others exist in most bacteria, plants and animals

Since net ribose synthesis in infected *E coli* was sharply reduced, it could be asked if the use of the phosphogluconate pathway had been similarly reduced. In the metabolism of glucose via this pathway, the first carbon, C_1, of glucose is selectively oxidized to CO_2, whereas in the metabolism of glucose via the Embden-Meyerhof pathway this carbon atom ends as the methyl carbon of pyruvate and lactate, a position in which its oxidation to CO_2 is minimal (Cohen, 1954, Wood, 1955). If glucose-1-C^{14} were offered growing bacteria, it was found that 22 per cent of the total glucose carbon used appeared as CO_2, but that 38 per cent of the C_1 appeared in this form During growth then the phosphogluconate pathway could account for a maximum of 38 per cent and a minimum of 16 per cent of the glucose used During infection, although total CO_2 production was unchanged, the $C^{14}O_2$ was sharply reduced, indicating a reduction in the utilization of the phosphogluconate pathway and a shift of some glucose to another pathway of glucose metabolism (Cohen, 1951b). It has been shown most recently that under such conditions a far greater proportion of the ribose produced in infected cells also arises from pathways other than the phosphogluconate pathway (Loeb and Cohen, 1958 in preparation).

Did virus infection directly inhibit enzymes of the phosphogluconate pathway? Disruption of infected cells revealed that the two initial enzymes of this pathway, the dehy-



Two alternative paths of glucose utilization present in *E coli* and in most other cells

have not been clearly resolved for any virus, their analysis has begun

A most important approach has involved the use of isotopically labeled virus Although most of the significant studies in this area have been performed in phage systems, similar studies have begun with animal virus systems The earliest experiments employed virus labeled in protein or nucleic acid to determine the general patterns of utilization of viral substance. Using P^{32}-labeled T_6, it was found first that from 30 to 40 per cent of the isotope subsequently appeared in material identified as progeny phage (Putnam and Kozloff, 1950). This result was generally confirmed with N^{15} as well as using T-even and T-odd phages, as summarized by Kozloff (1953), with the additional observation that significant amounts of DNA-P^{32} are rendered acid-soluble and hence are of low molecular weight (Lesley et al 1951) It was demonstrated that such a degradation is stimulated markedly under conditions of multiple infection and superinfection with the T-even phages and appears to be related to the "activation" of deoxyribonuclease occurring in infection with T-even phages (French et al, 1952, Graham, 1953) Very little degradation of viral protein occurred under comparable conditions (Kozloff, 1952a) and, as French (1954) subsequently showed, only 2 per cent of the lysine-C^{14} in labeled parent appeared in the progeny Thus, significant amounts of only viral nucleic acid appeared in the progeny.

Watson and Maaløe (1953), controlling phage adsorption to minimize superinfection breakdown and reducing readsorption of progeny to debris, raised the P^{32} or purine base transfer figures to about 50 per cent for T-odd and T-even phages This transferred substance was found predominantly in the early formed phages It was also found, as reported earlier by Kozloff (1952b) as well, that damaged or genetically different phages incapable of participating in multiplication nevertheless contributed their phage DNA to progeny as well In an earlier experiment (Maaløe and Watson, 1951), it was shown that progeny containing 30 per cent of their P^{32} from parental transfer also transferred only 30 per cent of their P^{32} to a second generation and it was concluded that the transfer of parental virus P and N was largely independent of genetically active units (Maaløe and Watson, 1951; Kozloff, 1952b).

In the experiment of Hershey and Chase (1952) on the selective penetration of phage DNA into the infected bacterium, the basis of the exclusion of viral protein was clearly shown At least 85 per cent of the viral protein which may be selectively labeled with S^{35} existed in the protein outer coat of the T-even phages S^{35} or P^{32} T_2 was adsorbed to *E coli*, and the infected cells were suspended in a medium low in electrolytes and spun in a Waring blendor, Strippable S^{35} was separated from the infected cells, still competent to yield phage, by low-speed centrifugation It was found that 80 per cent of viral S^{35} and amino acids and only 20 per cent of the DNA were in the supernatant fluid. Most viral protein then was in the disposable extracellular syringe while the viral DNA was injected and became intracellular Hershey (1955) has since found that up to 3 per cent of the viral protein is injected into the bacterium with the DNA, its function is unknown, and it is not transferred to the protein of viral progeny.

Similar phenomena of the attachment of the protein to the outer cell wall and the injection of DNA have been shown for T_5 (Lanni, 1954) and T_1 (Christensen and Tolmach, 1955) In the former instance, the attachment of phage protein and its detachment by the blending technic were followed by testing for the specific complement-fixing antigens of T_5

In general terms and as is discussed in greater detail in Chapter 7, only the phage DNA appears to transfer genetic information to the infected bacterium and, in the virulent systems studied, this code or set of codes replaces those of the bacterium whose nuclei are destroyed following infection In comparable transfer experiments with a lysogenic system of the temperate phage P_1 infecting Shigella dysenteriae (Goodgal, 1956), it was shown that almost all of the phage DNA is transferred to the bacterium Furthermore, the P^{32} was conserved to form a stable bacterial DNA component in the now lysogenic organism and was retained in subsequent divisions. In this instance, since multiple infection permitted the retention of the DNA of

fected by the T-even phages, the essential hydroxymethylating enzyme is not in uninfected bacteria or in phage, appears only on T-even phage infection and is associated with protein synthesis after the injection of viral DNA (Flaks and Cohen, 1957, 1958). Present data suggest that fragments of phage DNA containing the unique pyrimidine may induce the synthesis of the essential hydroxymethylase.

Reference has been made to respiration in infected tobacco plants and the changes appearing with increased translocation of substrates within the plants. Many metabolic alterations are known in virus-infected plants, these encompass the appearance of new products, increased amounts of old substances, and marked shifts in the content of certain enzymes and systems often found associated with chloroplasts (Wynd, 1942, 1943, Bawden, 1950). At the present time, it is not possible to relate these effects clearly to defined steps in the production of the plant viruses.

Of some interest is the report of the secretion of an extracellular α-amylase by virus-induced tumor tissue of the root of Rumex acetosa (Nickell and Brakke, 1954). The release of the enzyme was not due to cellular breakdown and could not be detected in cultures of normal root tissues. The problems of the origin of the enzyme seem to be similar in some respects to those posed by the Klebsiella system.

In the study of animal cells infected by many types of virus, e.g., yellow fever, lymphocytic choriomeningitis, etc., Bauer (1949) has reported the phenomenon of an apparent increase of xanthine oxidase. This has also been observed for influenza virus-infected tissue whose ability to oxidize many other substrates is virtually unchanged (Sellers and Jann, 1954). The causal relations of this change are not known, but it might be imagined that in virus-infected cells the metabolism of the nucleic acids is deranged in such a way as to release purines which induce the production of enzyme.

As noted above, the study of biochemical changes in cells infected in tissue culture has been begun. However, few satisfactory direct analyses of such cultures have been reported. Although Kovacs (1956a, b) has made many analyses of normal and poliomyelitis-infected cultures and has stated that infected cells show a marked drop in acid and alkaline phosphatase levels, it is not possible to correlate the stage of infection with the reported enzyme content of the cells.

One of the most elegant attempts to correlate biochemical events with virus infection was made in the study of the pathogenesis of poliomyelitis. The effect of infection obviously produces cell damage, e.g., chromatolysis of Nissl substance without significant mitochondrial damage. However, the inability to analyze infected cells as such obscured the possible significance of direct analyses and led to an effort to simulate these effects (chromatolysis) by sectioning peripheral nerves. Then it was discovered that during this type of induced chromatolysis the damaged cell is refractory to virus infection (Howe and Bodian, 1941). Thus it became possible to study the enzymatic changes coincident with the virus-resistant state in degenerating and regenerating cells (Gersh and Bodian, 1943; Bodian and Mellors, 1945, Howe and Mellors, 1945; Howe and Flexner, 1947, Bodian and Mellors, 1947). Although such studies were partially extended in the cytochemical work of Hyden (1947), their early promise has not been pursued.

SOME APPROACHES TO PROBLEMS OF DUPLICATION AND INHERITANCE

Several powerful and ingenious technics have been employed to explore the questions of the fate of infecting viral substance and of the mechanism of transfer of genetic information. The approaches to the latter aspect of the problem have attempted to identify the minimal structure responsible for coding inheritable characters by the addition to cells of purified viral fractions, e.g., nucleic acids, and examining for the production of virus. Such a study is directed primarily to the analysis of the division of labor in a virus particle.

In the experiments described below, the problem to be explored is that of the material coupling between the transfer of the code and the multiplication of viral substance. Is the code used and discarded or destroyed during utilization? Is the information transferred to a new code in a non-nucleic acid intermediate? Is the infecting nucleic acid self-duplicating? Although these and many other questions

sensitive photographic emulsions The particles formed tracks emanating from the site of deposition of the P^{32} containing fragment. From a knowledge of the half lifetime of P^{32} the specific activity of the P of the phage, and the number of individual tracks emanating from a star observed microscopically in a nuclear emulsion, as presented in Figure 29, it is theoretically possible to calculate the number of P atoms in the source of the star Ghosted phage particles were reported to contain two types of DNA, one of which was about 40 per cent the molecular weight of the total DNA of the phage (ca 45 million) whereas the remainder, undetected by this technic, consisted of smaller fragments (ca 8 million molecular weight) Parenthetically, it may be added that phage DNA has been reported to be separable on columns into two chemically distinct fractions (Brown in Levinthal and Thomas, 1957), but not so separable in ultracentrifugation (Cohen, 1957b)

Using the star technic, one large fragment equivalent to about 40 per cent of the phage DNA is reported to be transferred to the bacterium and a fragment of half this size is found in the first generation progeny In the second and subsequent generations derived from these first-generation progeny the 20 per cent fragment is transferred intact Thus, this result appears to be consistent with the conservation of a single thread template as suggested in the Watson-Crick hypothesis concerning the self-duplicability of DNA That the material continuity of such a fragment is in fact associated with the transfer of inheritable characters has yet to be proved

Another test of the transfer of parental P^{32} arose from the observation that phage containing highly radioactive P^{32} die at a rate proportional to the number of radioactive atoms which they contain, as a function of the decay of P^{32} to S^{32} (Hershey et al, 1951) Exploration of this phenomenon as a measure of the P^{32} contained in progeny by Stent and colleagues (Delbruck and Stent, 1957) has also revealed transferred DNA to be contained in a few phage particles This DNA tended also to be conserved However, the data of Stent et al are not entirely in agreement with the hypotheses of Levinthal (see Hershey and Burgi, 1956, Delbruck and Stent, 1957)

Nevertheless, in addition to the potential biologic importance of the Levinthal experiment, the star technic represents a fundamental contribution to the methodology of study of the fate of very large single biologically active molecules Its application in this system points up the technical advantages of using viral systems containing minimal numbers of genetically active molecules in the study of the chemical basis of inheritance not only in contrast with the uninterpretable systems of higher organisms but also vis-à-vis the transforming systems in pneumococci, etc, in which the unfractionated genetic complement contains hundreds of DNA molecules

Once nucleic acid has entered cells, several technics may be used to determine its subsequent fate A powerful tool has been that of irradiating infected cells with ultraviolet light or x-ray and comparing the rates at which free virus and virus-infected centers lose the ability to produce virus The technic was first applied to phage systems (Luria and Latarjet, 1947, Latarjet, 1948, Benzer, 1952) and was used to show that during the first 7 minutes the infectious unit of T_2 possessed the same sensitivity within the cell as it did before infection Thus, in this interval, the injected DNA neither multiplies nor transfers its information to less-sensitive units T_7-bacterium completes maintained the sensitivity of free T_7 through 4 minutes of a short latent period After 7 minutes, the sensitivity of T_2-bacterium complexes fell rapidly, whereas T_7 complexes essentially retained their sensitivities after the multiple unit phase of the curve was passed Thus, the later phases of the multiplication of these viruses appeared to be significantly different

With the plant viruses, it is possible to compare the sensitivity of the free viruses with that of their infectious nucleic acids It has been found that the sensitivities of the nucleic acids of different strains of tobacco mosaic virus were identical, although the intact viruses possessed different sensitivities to ultraviolet radiation (Siegel et al, 1956) Irradiation of tobacco leaves infected by these nucleic acids showed that the infectious centers also were as sensitive as the nucleic acids (Siegel et al, 1957). However, immediately after infection with intact virus, the infectious

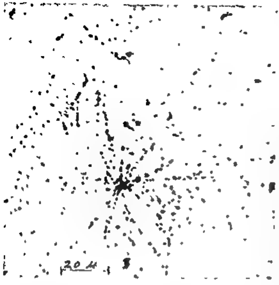


FIG. 29. A photomicrograph of a star produced in sensitive emulsion by the emission of β-particles from a site of decaying P32 (Levinthal, C, and Thomas, C A., 1957, The molecular basis of genetic recombination in phage *in* McElroy, W, and Glass, B [eds], Chemical Basis of Heredity, pp 735-743, Baltimore, Johns Hopkins Press)

only one phage particle, it might be inferred that the bacterial chromosome had only one site available for prophage formation

Comparable experiments on bacterial transformations have been performed recently with P^{32}-DNA (Lerman and Tolmach, 1957; Fox, 1957) and the percentage of transformations is found to be directly proportional to DNA uptake The transformation of a single character appears to require the adsorption of about 500 molecules of DNA, *i e*, in these cases the equivalent of an entire bacterial genome which would have a single unit of effective transforming DNA

As noted earlier, influenza virus can be labeled with P^{32} present in phospholipid and nucleic acid If chorio-allantoic membrane is analyzed 90 minutes after infection with P^{32}-labeled virus, some P^{32} is found in the low-salt extractable nucleoprotein of the infected membrane and some lipid-P^{32} is degraded to form nonsedimentable material (Hoyle and Frisch-Niggemeyer, 1955) Of considerable interest is the presence of a large proportion (25%) of membrane-bound P^{32} in the cell nucleus in a form extractable by a nucleoprotein solvent, *i e*, M NaCl The authors interpreted this data to signify that on entry into the host cell the influenza virus particle is broken down Phospholipid was destroyed, and the nucleoprotein released, at least part of its nucleic acid, to become associated with the cell nucleus.

Hoyle and Finter (1957) have studied the fate of infecting influenza virus labeled with radioactive methionine In this case S^{35} does not become associated with the cell nucleus. Most of the S^{35} (70%) remains associated with particles of size comparable with virus, which are thought to be envelope protein, since none is present in infectious virus Some 10 to 20 per cent of the S^{35} is recovered as low molecular fragments, suggested to be derived from the degradation of the viral nucleoprotein released from viral envelope.

In the hypothesis of Watson and Crick (1953) the two threads of the helical coil of DNA are paired by complementary bases in such a way that each single thread may organize the duplication of a complementary thread The successive fragmentation of the DNA in parent to progeny experiments implied the nonconservation of either thread of the template. However, it has been suggested that the figure of up to a 50 per cent yield of P^{32} in experiments involving isotope transfer from parent to progeny arise from the technical and biologic difficulties of (1) preparing completely viable phage preparations, (2) superinfection breakdown, (3) early lysis of infected cells with loss of intermediate precursors, (4) readsorption of released virus on bacterial debris Hershey and Burgi (1956) have supported this view experimentally As indicated earlier, they have also obtained evidence that the transfer of C^{14}-labeled bases from parental virus occurred in the same ratio as they existed in phage DNA and that a dilution of any parental base could not be effected by competition with exogenously supplied thymidine. Thus, viral DNA does seem to be transferred in large polynucleotide fragments, contrary to the earlier results reported above

An ingenious technic was devised to test this possibility (Levinthal, 1956, Levinthal and Thomas, 1957a) The β-particles emitted by decaying P^{32} in DNA labeled with this isotope were detected by exposure to very

numbers of reactions culminating in DNA replication. Such a rate could be determined for example by the number of enzyme molecules making available a critical deoxyribotide triphosphate

Studies of the sizes of the DNA and protein-precursor pools have revealed that the former is considerably greater, suggesting that the DNA need not be formed within an intact phage skin (Hershey et al., 1954) If a large pool of T_2 viral DNA is permitted to be synthesized in chloramphenicol-inhibited bacteria, this DNA can be encased subsequently by phage protein which the infected cells make when chloramphenicol is removed from the system (Hershey and Melechen, 1957) However, the structural features of the molecules within this intracellular DNA pool are far from clear, since it has not been demonstrated that this material is indeed free despite the existence of some electrophoretic (Singer et al., 1952) and ultracentrifugation data (Watanabe et al., 1955) on extracts of infected cells

Even if phage DNA is self-duplicating, this experiment does not mean that the pool of free DNA directs subsequent protein synthesis, since this may, in fact, be determined by the synthesis of other components in the early phase of infection before the synthesis of phage protein and DNA normally begin (Barry, 1954, Maaløe and Symonds, 1953) Indeed, if infected cells are irradiated to block DNA synthesis after it has already begun, the synthesis of phage antigen continues, implying that the protein-synthesizing mechanism has, in fact, been set by substances more resistant to radiation than is DNA (Watanabe, 1957) At the outset of infection, the functions of DNA duplication and synthesis of phage protein are equally sensitive to radiation of the infected cell Thus, the code for specific protein synthesis seems to be transferred to something other than DNA.

If the synthesis of viral protein and nucleic acid do occur separately, as has been suggested for the bacterial and plant virus systems, the problems of assembly are critical reactions Although the assembly of nucleic acid and protein to form tobacco mosaic virus appears to occur spontaneously in an adequately interdigitated way, as described in Chapter 2, it is very difficult to conceive of this occurring in a similarly simple reaction in the production of intact phage, although such a phenomenon is inferrable from the many experiments reported in this chapter, e.g., phenotypic mixing, coating of phage DNA, etc.

At the present time, the kinetics of development of influenza virus is also consistent with the possibility of a separate development of hemagglutinin and soluble nucleoprotein which are coated together shortly before viral release On the other hand, the development of vaccinia virus viewed in the electron microscope suggests for the time being a more closely integrated synthesis of DNA within a protein shell At present, there is as yet too little known about late stages of assembly in any viral systems to permit a serious comparison of their mechanisms

A UNIQUE CASE OF EXTREME PARASITISM-HYDROXYMETHYLCYTOSINE AND T-EVEN PHAGE SYSTEMS

It is the purpose of the author in this section to record biochemical phenomena for which there are no known biochemical precedents in normal systems Although the generalization of past experience and approaches can facilitate progress, the research worker must also be prepared for new and different phenomena, which he must be prepared to pursue without existing precedent

In the experiments with T-even phage systems presented above, it was determined that the patterns of nucleic acid metabolism and protein and enzyme synthesis were markedly changed by infection This occurred without apparent changes in energy supply and respiration, nevertheless, the infected cells developed a profound alteration in the balance of metabolic pathways used to metabolize glucose However, this alteration in the mechanism of glucose utilization was controlled at a level of metabolism other than that of the early metabolism of glucose. All of these phenomena can be interpreted in terms of the net production of viral DNA with a concomitant cessation of synthesis of bacterial DNA and RNA How can the shift to the increased accumulation of this substance be explained?

A simple explanation stems from the dis-

centers were approximately as sensitive as the intact virus, i e, less sensitive than were the infectious centers produced by the respective nucleic acids These sensitivities remained constant for hours, the duration of this period depending on the virus used It appeared that free nucleic acid multiplied immediately, whereas the viruses within virally induced infectious centers, but not nucleate induced centers, multiplied only after shedding their protein coats, a process taking some hours. Comparable studies have not yet been performed in animal virus systems

In studying the intracellular fate of these viral nucleic acids and the problem of nucleic acid metabolism in general, attempts have been made to apply ribonuclease and deoxyribonuclease The study of the effects of these enzymes has not yet produced clear results with phage systems (Kleczkowski and Kleczkowski, 1954, Fraser and Mahler, 1957; Jerne and Maaløe, 1957). However, ribonuclease has yielded interesting results with RNA virus systems. For example, when ribonuclease is infiltrated into tobacco leaves, this treatment inhibits multiplication of tobacco mosaic virus even if infection had occurred up to 2 hours before addition of the enzyme (Hamers-Casterman and Jeener, 1957) This enzyme similarly inhibits the multiplication of influenza virus in chorio-allantoic membrane in tissue culture (LeClerc, 1956) Again, this effect is manifest only within 2 hours after infection The inhibitory effect of the enzyme on virus multiplication was observed without change in the incorporation of C^{14}-phenylalanine, although the incorporation of C^{14}-adenine was inhibited markedly

It would appear that nucleic acid initiates specific duplication in these systems but how is this effected? In the phage systems does DNA duplicate itself with the production of RNA and viral protein as a concomitant by-product? Or does it compel the synthesis of RNA which directs viral protein synthesis, with one of these then directing DNA synthesis? Or does the DNA compel, with the concomitant by-product RNA, the synthesis of protein, which then directs DNA synthesis? It is very difficult to say at present. As noted earlier, it is known in the T-even phage systems that a prior synthesis of protein containing deoxycytidylate hydroxymethylase is required before DNA synthesis can occur. However, other phage systems also show this requirement for protein synthesis despite the absence of hydroxymethyl cytosine in these phage DNA's so that it may be supposed that early protein syntheses of unknown function are required in all systems

That this protein may have some genetic function has been suggested as a result of the work of Stent (1955) and Stent and Fuerst (1956) Using highly radioactive virulent or temperate P^{32}-phage to infect bacteria, phage development was allowed to proceed for various times before the infected bacteria were frozen and stored. Immediately after infection the ability of the infected cells to produce phage could be shown to decrease as a function of radioactive decay of P^{32}. However, after a few minutes of intracellular phage development the ability of these cells to produce phage became independent of radioactive decay. One hypothesis to explain this result would be that the code for phage production was transferred to moieties free of P, perhaps to protein (Stent, 1955) In support of this concept are the experiments of Tomizawa and Sunakawa (1956) who have demonstrated that, despite the synthesis of viral nucleic acid, the inhibition of protein synthesis in chloramphenicol-inhibited infected bacteria prevents a change in the radiation sensitivity of infected cells

On the other hand, as described in Chapter 7 in greater detail, other workers have supposed that viral DNA is self-duplicating in the model of Watson and Crick Phage antigens do not exist in uninduced lysogenic bacteria (Miller and Goebel, 1954) and in conjunction with the experiment of Goodgal (1956) this may be suggested to signify that prophage is naked viral DNA which multiplies in this form As described by Luria (1951) and Tanami (1957), the frequency distribution of spontaneous phage mutant clones suggests that the replication of viral genetic units proceeds in such a way that replicas themselves may serve as patterns for additional replications This is evidently consistent with the concept of viral DNA as a self-duplicating unit Furthermore, this picture is not inconsistent with the observed linear production of phage DNA, since the latter may only reflect some rate limiting step in the large

esterase activity (Cohen, 1953). Subsequently, it was found that such enzyme-resistant fragments contained derivatives of HMC in which glucose was attached through glycosyl linkage to the hydroxymethyl group (Volkin, 1954; Sinsheimer, 1954, Cohen, 1956, Jesaitis, 1957) Thus, this new substituent renders these viral DNA's more resistant to types of enzymatic activity that completely degrade host DNA

It has been shown that the glucose contents of the T-even phages are in the order $T_2r^+ < T_4r^+ < T_6r^+$, although their HMC and other base contents are identical. The glucose to HMC ratios in these viruses are 0 8, 1 0 and 1 6, respectively Preparations of the "r" phages have been found to contain more glucose than do homologous r^+ phages (Cohen, 1956), however, it has not been proved that such results do not arise from contamination of these more difficultly purifiable phages A correlation has been observed between the infectivity of a T-even phage and the glucose content of its DNA (Streisinger and Weigle, 1956, Sinsheimer, 1956) A new type of inheritance has been observed by these workers in which the glucose content of T_2 produced in a bacterium simultaneously infected with T_4 is raised to that of T_4 Such particles continue to multiply as T_2, although maintaining the glucose content of T_4 Thus, gross glucose content does not entirely determine T_2ness, although it certainly establishes some properties of wild type T_2 particles

On treatment of T-even phage DNA with deoxyribonuclease and phosphodiesterase, about 25 per cent of the HMC is liberated as mononucleotides T_2-DNA liberates a mixture of nonglucosylated and monoglucosylated nucleotides, T_4-DNA liberates only monoglucosylated nucleotides, and T_6-DNA releases a mixture of nonglucosylated and diglucosylated nucleotides (Jesaitis, 1957) A smaller amount of monoglycosylated nucleotides and a relatively larger amount of diglucosylated HMC-containing dinucleotide have also been found in the latter instance (Cohen and Lichtenstein, 1958) Thus, glucose modifies the HMC sufficiently to suggest that the insertion of particular types of HMC nucleotides into the DNA chain probably does confer genetic specificity upon this chain However, it is not possible to say at present how glucosylation occurs or how the selection of particular HMC nucleotides is effected In addition, it might be asked if the presence of diglucosyl HMC nucleotides in T_6 does not induce the mechanism for adding a second glucose to a monoglycosylated HMC nucleotide and if indeed a similar mechanism does not account for conferring the glucose contents of T_4 on T_2 particles during mixed infections

Thus, HMC appears to play at least 3 major roles in the multiplication of T-even phages:

1 It helps to explain the parasitism and trapping mechanism which diverts bacterial metabolism to making virus

2 It provides a point of attachment for glucosyl moieties which confer stability to DNA by protecting phosphodiester linkages containing HMC nucleotides

3 Various glycosylated states of HMC nucleotides modify the order of the polynucleotide chain, in part determining speciation among the T-even viruses

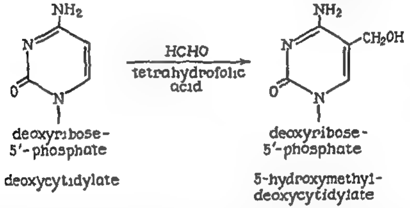
CONCLUSION

Despite the fact that historical accident focused a large portion of biochemical study on the unique T-even phages, their extreme virulence and other special properties facilitated the development of a biochemical methodology in which a large number of biochemical technics were applied successfully to the analysis of virus-cell interactions and the steps in virus multiplication As we have seen, studies on these unique systems have contributed to biochemistry in general, providing insight into problems of the metabolism of carbohydrates, of 1-carbon fragments, and of polymer structure and biosynthesis, with particular reference to the chemistry of duplication and inheritance. The methodology which has been developed has already been applied in part to some animal and plant virus-cell systems and will be applicable to many others after their elementary biologic properties will have been established Until the biochemical behavior of the infected animal cell, the unit of viral disease, is clarified, it does not appear fruitful to consider in detail the problem of the biochemical patho-

covery of a new and unique pyrimidine, 5-hydroxymethylcytosine (HMC) in the DNA of the T-even phages (Wyatt and Cohen, 1953). The normal pyrimidine, cytosine, although present in bacterial DNA and RNA, is not present in the viral DNA In fact, the cytosine deoxyribotide of bacterial DNA is converted to the HMC nucleotide of viral DNA The reaction involves the hydroxymethylation of cytosine deoxyribotide in the presence of tetrahydrofolic acid to form hydroxymethylcytosine deoxyribotide (Flaks and Cohen, 1957)

T-even phage and increases to a maximum 15 minutes after infection. It may be noted that the rate of viral DNA synthesis is maximal between 7 to 10 minutes, and more enzyme continues to be produced than is necessary to attain this maximal rate. Infection in the presence of 5-methyl tryptophan prevents its appearance; subsequent addition of tryptophan then results in the production of enzyme. Thus, the production of enzyme seems to require protein synthesis

The production of the enzyme need not be tied to the duplication of DNA and virus



The reaction is irreversible, and the enzyme is present in excess, permitting a trapping of all the deoxycytidylate formed and rendering it unavailable for synthesis of normal bacterial DNA. It may be supposed, although not yet proved, that the trapping action may extend to cytosine ribotide essential for the production of bacterial RNA, if the conversion of deoxycytidylate from cytidylate is hastened thereby The deficiency of cytosine derivatives in preventing the synthesis of bacterial nucleic acids may be expected to prevent the gross production of bacterial polymers, particularly proteins, associated with the nucleic acids

Why does this trapping action not occur during normal growth? The deoxycytidylate hydroxymethylase is not present in normal bacteria nor can it be released by various types of disintegration of normal bacteria It cannot be found in bacteria infected in the absence of HMC-containing DNA, e.g., T_1 On the other hand, it is not detectable within T-even phages The enzyme begins to appear within 0 to 3 minutes after infection by a

duplication Thus, phage extensively inactivated by ultraviolet light will induce essentially normal formation of enzyme under conditions in which virus does not multiply (Flaks and Cohen, 1958) It may be supposed that bits of virus DNA, possibly from a fraction which does not serve as a template for virus multiplication, act as inducer It had been found earlier that protein synthesis continued under conditions of infection with ultraviolet-infected phage (Cohen, 1951) although DNA synthesis need not continue. In this unique system, it appears possible that a portion of the DNA may induce an enzyme essential to the production of a component essential to the duplication of genetic material.

The existence of the hydroxymethyl group provides a point of attachment for additional substituents. Fractions of the DNA of the T-even phages have been extraordinarily resistant to phosphoesterases These fractions proved to contain HMC, and a preliminary acid hydrolysis was necessary to make HMC-containing nucleotides available to phospho-

esterase activity (Cohen, 1953) Subsequently, it was found that such enzyme-resistant fragments contained derivatives of HMC in which glucose was attached through glycosyl linkage to the hydroxymethyl group (Volkin, 1954; Sinsheimer, 1954, Cohen, 1956; Jesaitis, 1957). Thus, this new substituent renders these viral DNA's more resistant to types of enzymatic activity that completely degrade host DNA

It has been shown that the glucose contents of the T-even phages are in the order $T_2r^+ < T_4r^+ < T_6r^+$, although their HMC and other base contents are identical The glucose to HMC ratios in these viruses are 0 8, 1 0 and 1 6, respectively Preparations of the "r" phages have been found to contain more glucose than do homologous r^+ phages (Cohen, 1956); however, it has not been proved that such results do not arise from contamination of these more difficultly purifiable phages A correlation has been observed between the infectivity of a T-even phage and the glucose content of its DNA (Streisinger and Weigle, 1956; Sinsheimer, 1956). A new type of inheritance has been observed by these workers in which the glucose content of T_2 produced in a bacterium simultaneously infected with T_4 is raised to that of T_4 Such particles continue to multiply as T_2, although maintaining the glucose content of T_4 Thus, gross glucose content does not entirely determine T_2ness, although it certainly establishes some properties of wild type T_2 particles

On treatment of T-even phage DNA with deoxyribonuclease and phosphodiesterase, about 25 per cent of the HMC is liberated as mononucleotides T_2-DNA liberates a mixture of nonglucosylated and monoglucosylated nucleotides, T_4-DNA liberates only monoglucosylated nucleotides, and T_6-DNA releases a mixture of nonglucosylated and diglucosylated nucleotides (Jesaitis, 1957) A smaller amount of monoglycosylated nucleotides and a relatively larger amount of diglucosylated HMC-containing dinucleotide have also been found in the latter instance (Cohen and Lichtenstein, 1958) Thus, glucose modifies the HMC sufficiently to suggest that the insertion of particular types of HMC nucleotides into the DNA chain probably does confer genetic specificity upon this chain However, it is not possible to say at present how glucosylation occurs or how the selection of particular HMC nucleotides is effected In addition, it might be asked if the presence of diglucosyl HMC nucleotides in T_6 does not induce the mechanism for adding a second glucose to a monoglycosylated HMC nucleotide and if indeed a similar mechanism does not account for conferring the glucose contents of T_4 on T_2 particles during mixed infections

Thus, HMC appears to play at least 3 major roles in the multiplication of T-even phages·

1 It helps to explain the parasitism and trapping mechanism which diverts bacterial metabolism to making virus

2 It provides a point of attachment for glucosyl moieties which confer stability to DNA by protecting phosphodiester linkages containing HMC nucleotides.

3 Various glycosylated states of HMC nucleotides modify the order of the polynucleotide chain, in part determining speciation among the T-even viruses

CONCLUSION

Despite the fact that historical accident focused a large portion of biochemical study on the unique T-even phages, their extreme virulence and other special properties facilitated the development of a biochemical methodology in which a large number of biochemical technics were applied successfully to the analysis of virus-cell interactions and the steps in virus multiplication As we have seen, studies on these unique systems have contributed to biochemistry in general, providing insight into problems of the metabolism of carbohydrates, of 1-carbon fragments, and of polymer structure and biosynthesis, with particular reference to the chemistry of duplication and inheritance The methodology which has been developed has already been applied in part to some animal and plant virus-cell systems and will be applicable to many others after their elementary biologic properties will have been established. Until the biochemical behavior of the infected animal cell, the unit of viral disease, is clarified, it does not appear fruitful to consider in detail the problem of the biochemical patho-

genesis of viral disease in an intact multicellular organism Indeed, questions relating to this problem have not yet even been posed in suitable forms; in general, it is difficult to run before learning to walk.

REFERENCES

Ackermann, W W, 1951, Concerning the relation of Krebs cycle to virus propagation, J Biol Chem 189, 421-428

———, 1954, Intracellular sites important to the development of animal viruses, in The Dynamics of Virus and Rickettsial Infections, Hartman, F W, Horsfall, F L, and Kidd, J G (eds), pp 101-110, New York, Blakiston

Ackermann, W W, and Johnson, R B, 1953, Some energy relations in a host-virus system, J Exper Med 97, 315-322

Ackermann, W W, and Kurtz, H, 1952, The relation of herpes virus to host cell mitochondria, J Exper Med 96, 151

Ackermann, W W, and Maassab, H F, 1954, Growth characteristics of influenza viruses the influence of sulfonic acid, J Exper Med 99, 105-117

———, 1955, Growth characteristics of influenza virus II Biochemical differentiation on stages of development J Exper Med 102, 393-402

Ada, G L, and Perry, B T, 1955, Infectivity and nucleic acid content of influenza virus, Nature, London 175, 209-210

Adams, M H, 1954, Abortive infections with viruses, in The Dynamics of Virus and Rickettsial Infections, Hartman, F W, Horsfall, F L, and Kidd, J G (eds), pp 86-93, New York, Blakiston

Adams, M H, and Park, B H, 1956, An enzyme produced by a phage-host cell system II The properties of the polysaccharide depolymerase Virology 2, 719-736

Allen, E H, and Bovarnick, M R, 1957, Association of reduced diphosphopyridine nucleotide cytochrome c reductase activity with meningopneumonitis virus, J Exper Med 105, 539-547

Anderson, T F, 1945, On a bacteriolytic substance associated with a purified bacterial virus, J Cell & Comp Physiol. 25, 1-15

———, 1949, The reactions of bacterial viruses with their host cells, Botan Rev 15, 464-505

Barner, H D, and Cohen, S S, 1954, The induction of thymine synthesis by T_2 infection of a thymine requiring mutant of *Escherichia coli*, J Bact 68, 80-88

Barrington, L F, and Kozloff, L M, 1956, Action of bacteriophage on isolated host cell walls, J Biol Chem 223, 615-627

Barry, G T, 1954, A study of the antigenicity of T_3 and T_4 coli-dysentery bacteriophages during the vegetative stage of development, J Exper Med 100, 163-180

Basler, E, and Commoner, B, 1956, The effect of tobacco mosaic virus biosynthesis on the nucleic acid content of tobacco leaf, Virology 2, 13-28

Bauer, D J, 1949, Multiplication of the animal viruses, Nature, London 164, 767-771

Bawden, F C, 1950, Plant Viruses and Virus Diseases, Waltham, Mass, Chronica Botanica Co

Benzer, S., 1952, Resistance to ultraviolet light as an index to the reproduction of bacteriophage, J Bact 63, 59-72

———, 1957, The elementary units of heredity, in The Chemical Basis of Heredity, McElroy and Glass (eds), pp 70-93, Baltimore, Johns Hopkins Press

Bergold, G H, and Wellington, E F., 1954, Isolation and chemical composition of the membranes of an insect virus and their relation to the virus and elementary bodies, J Bact 67, 210-216.

Bertani, G, 1958, Lysogeny, in Advances in Virus Research, vol 5, pp 151-193, New York, Acad Press

Bertani, L E, 1957b, The effect of the inhibition of protein synthesis on the establishment of lysogeny, Virology 4, 53-71

Beveridge, W I B, and Burnet, F M, 1946, The cultivation of viruses and rickettsiae in the chick-embryo Med Res Counc Spec Report, Ser No 256, London

Blank, H, 1946, Radioactive isotopes for the study of animal viruses, Tr New York Acad Sc, ser 2, 18, 243-248

Bloch, D P, and Godman, G C, 1957, A cytological and cytochemical investigation of the development of the viral papilloma of human skin, J Exper Med 105, 161-176

Bloch, D P, Morgan, C, Godman, G C, Howe, C, and Rose, H M, 1957, A correlated histochemical and electron microscopic study of the intranuclear crystalline aggregates of adenovirus (RI-APC virus) in DeLa cells J Biophys Biochem Cytology 3, 1-8

Bodian, D, and Mellors, R C, 1945, The regenerative cycle of motoneurons, with special reference to phosphatase activity, J Exper Med 81, 469-488

———, 1947, Decrease of phosphocreatine in regenerating neurons, J Biol Chem 167, 655-661

Bolton, E, 1954 Biosynthesis of nucleic acid in *Escherichia coli*, Proc Nat Acad Sc 40, 764-772

Bovarnick, M R, 1956, Phosphorylation accompanying the oxidation of glutamate by the Madrid E strain of typhus rickettsiae, J Biol Chem 220, 353-361

Boyer, G S, Leuchtenberger, C, and Ginsberg, H., 1957, Cytological and cytochemical studies of HeLa cells infected with adenoviruses, J Exper Med 105, 195-216

Breitenfeld, P M, and Schafer, W, 1957 The formation of fowl plague virus antigens in infected cells, as studied with fluorescent antibodies, Virology 4, 328-345

Brenner, S, 1957 Genetic control and phenotypic mixing of the adsorption cofactor requirement in bacteriophages T_2 and T_4, Virology 3, 560-574

Burton, K, 1955, The relation between the synthesis of deoxyribonucleic acid and the synthesis of protein in the multiplication of bacteriophage T_2, Biochem J 61, 473-483

Christensen, J R., and Tolmach, L J., 1955, On the early stages of infection of *Escherichia coli* B by bacteriophage T_1, Arch Biochem 57, 193-207

Cohen, S S, 1944, Cytoplasmic particles of chorio-allantoic membrane and their relations to purified preparations of influenza virus, Proc Soc Exper Biol & Med. 57, 358-360

———, 1947, The synthesis of bacterial viruses in infected cells, Cold Spring Harbor Symp Quant Biol *12*, 35-49

———, 1948a, The synthesis of bacterial viruses I The synthesis of nucleic acid and protein in *Escherichia coli* B infected with T_2r^+ bacteriophage J Biol Chem *174*, 281-293

———, 1948b, The synthesis of bacterial viruses II The origin of the phosphorus found in the desoxyribonucleic acids of the T_2 and T_4 bacteriophages J Biol Chem *174*, 295-303

———, 1949, Growth requirements of bacterial viruses, Bact Rev *13*, 1-24

———, 1951, Biochemical studies on multiplication of bacterial viruses, Fed Proc *10*, 585-590

———, 1951b, Utilization of gluconate and glucose in growing and virus-infected *Escherichia coli* Nature London *168*, 746-747

———, 1953, Studies on controlling mechanisms in the metabolism of virus-infected bacteria, Cold Spring Harbor Symp Quant Biol *18*, 221-235

———, 1954, Other pathways of carbohydrate metabolism, *in* Chemical Pathways of Metabolism, ed by Greenberg, D pp 111 213, New York Acad Press

———, 1955, Comparative biochemistry and virology, in Advances in Virus Research, vol 3, pp 1-48, New York, Acad Press

———, 1956, Molecular bases of parasitism of some bacterial viruses, Science *123*, 653-656

———, 1957b, Discussion of Hershey's paper in Cellular Biology, Nucleic Acids and Viruses (Special Publication), vol 5 pp 263-265, New York Acad Sciences

Cohen, S S, and Anderson, T F, 1946, Chemical studies on host-virus interaction I The effect of bacteriophage adsorption on the multiplication of its host, *Escherichia coli* [illegible] With an appendix giving some data on the composition of the bacteriophage, T_2 J Exper Med *84*, 511-523

———, 1946, Chemical studies on host-virus interactions II The chemical simulation of the interference phenomenon by 5-methyl tryptophan J Exper Med *84*, 525 533

Cohen, S S, and Arbogast, R, 1950, Chemical studies on host-virus interactions VIII The mutual reactivation of T_2r+ inactivated by ultraviolet light and the synthesis of desoxyribose nucleic acid J Exper Med *91*, 637-650

Cohen, S S and Fowler, C B, 1947, Chemical studies on host-virus interactions III Tryptophan requirements in the stages of virus multiplication in the *Escherichia coli*-T_2 bacteriophage system J Exper Med *85*, 771-784

———, 1948, Chemical studies in host-virus interactions V Some additional methods of determining nutritional requirements for virus multiplicati J Exper Med *87*, 275-282

Cohen, S S, Lichtenstein, J, Barner, H D a Green, M, 1957, Studies on the biosynthesis bacterial and viral pyrimidines IV Utilization pyrimidine bases and nucleosides by bacterial n tants J Biol Chem *228*, 611-621

Cohen, S S, and Roth, L, 1953, The phospl gluconate pathway of carbohydrate metabolism the multiplication of bacterial viruses, J Bact 490-495

Commoner, B, Lippincott, J A, Shearer, G Richman, E E, and Wu, J, 1956, Reconstitut of tobacco mosaic virus components, Nature, Lo don *178*, 767-771

Commoner, B, Schreiber, [illegible] L, and Dietz, P 1953, Relationships between tobacco mosaic vi biosynthesis and the nitrogen metabolism of host, J Gen Physiol 36, 807-830

Delbruck, M, and Bailey, W T, 1946, Indu mutations in bacterial viruses, Cold Spring Harl Symp Quant Biol *11*, 33-37

Delbruck, M, and Stent, G S, 1957, On the mech ism of DNA replication, in The Chemical Basis Heredity, McElroy, W, and Glass, B (eds), 699-736, Baltimore, Johns Hopkins Press

DeMars, R I, 1953, Chemical mutagenesis in b teriophage T_2 Nature, London *172*, 964

DeMars, R I, Luria, S E, Fisher H, and Levinth C, 1953, The production of incomplete bacter phage particles by the action of proflavine a the properties of the incomplete particles, A Inst Past *84*, 113-129

Doermann, A H, 1948, Intracellular growth of b teriophage, Carnegie Inst Washington Yrbk 176-182

———, 1952, The intracellular growth of bacter phages I Liberation of intracellular bacteriophi T_4 by premature lysis with another phage or w cyanide J Gen Physiol 35, 645-656

Doermann, A H, Chase M, and Stahl, F W, 19 Genetic recombination and replication in bacter phage, J Cell & Comp Physiol *45*, (Suppl 51-74

Dolby, D E, 1955, The effect of enzyme inhibit on the multiplication of T_2 bacteriophage, J G Microbiol *12*, 406-418

Dulbecco, R, 1952 Production of plaques in mo layer tissue cultures by single particles of an a mal virus, Proc Nat Acad Sc 18, 747-752

Dulbecco, R, and Vogt, M, 1953, Some problems animal virology as studied by the plaque techniq Cold Spring Harbor Symp Quant Biol *18*, 2 279

———, 1954, One-step growth curve of west equine encephalomyelitis virus on chicken emb cells grown in vitro and analysis of virus yie from single cells, J Exper Med 99, 183-199

Eagle, H, 1955, The specific amino acid requireme of a mammalian cell (strain L) in tissue cultu J Biol Chem *214*, 839-852

Eagle, H, and Habel, K., 1956, The *nutritional* quirements for the propagation of poliomyel

genesis of viral disease in an intact multicellular organism. Indeed, questions relating to this problem have not yet even been posed in suitable forms; in general, it is difficult to run before learning to walk

REFERENCES

Ackermann, W W, 1951, Concerning the relation of Krebs cycle to virus propagation, J Biol Chem 189, 421-428

———, 1954, Intracellular sites important to the development of animal viruses, in The Dynamics of Virus and Rickettsial Infections, Hartman, F W, Horsfall, F L, and Kidd, J G (eds), pp 101-110, New York, Blakiston

Ackermann, W W, and Johnson, R B, 1953, Some energy relations in a host-virus system, J Exper Med 97, 315-322

Ackermann, W W, and Kurtz, H, 1952, The relation of herpes virus to host cell mitochondria, J Exper Med 96, 151

Ackermann, W W, and Maassab, H F, 1954, Growth characteristics of influenza viruses the influence of sulfonic acid, J Exper Med 99, 105-117

———, 1955, Growth characteristics of influenza virus II Biochemical differentiation on stages of development J Exper Med 102, 393-402

Ada, G L, and Perry, B T, 1955, Infectivity and nucleic acid content of influenza virus, Nature, London 175, 209-210

Adams, M H, 1954, Abortive infections with viruses, in The Dynamics of Virus and Rickettsial Infections, Hartman, F W, Horsfall, F L, and Kidd, J G (eds), pp 86-93, New York, Blakiston

Adams, M H, and Park, B H, 1956, An enzyme produced by a phage-host cell system II The properties of the polysaccharide depolymerase Virology 2, 719-736

Allen, E G, and Bovarnick, M R, 1957, Association of reduced diphosphopyridine nucleotide cytochrome c reductase activity with meningopneumonitis virus, J Exper Med 105, 539-547

Anderson, T F, 1945, On a bacteriolytic substance associated with a purified bacterial virus, J Cell & Comp Physiol 25, 1-13

———, 1949, The reactions of bacterial viruses with their host cells, Botan Rev 15, 464-505

Barner, H D, and Cohen, S S, 1954, The induction of thymine synthesis by T_2 infection of a thymine requiring mutant of Escherichia coli, J Bact 68, 80-88

Barrington, L F, and Kozloff, L M, 1956, Action of bacteriophage on isolated host cell walls, J Biol Chem 223, 615-627

Barry, G T, 1954, A study of the antigenicity of T_3 and T_4 coli-dysentery bacteriophages during the vegetative stage of development, J Exper Med 100, 163-180

Basler, E, and Commoner, B, 1956, The effect of tobacco mosaic virus biosynthesis on the nucleic acid content of tobacco leaf, Virology 2, 13-28

Bauer, D J, 1949, Multiplication of the animal viruses, Nature, London 164, 767-771

Bawden, F C, 1950, Plant Viruses and Virus Diseases, Waltham, Mass, Chronica Botanica Co

Benzer, S, 1952, Resistance to ultraviolet light as an index to the reproduction of bacteriophage, J Bact 63, 59-72

———, 1957, The elementary units of heredity, in The Chemical Basis of Heredity, McElroy and Glass (eds), pp 70-93, Baltimore, Johns Hopkins Press

Bergold, G H, and Wellington, E F, 1954, Isolation and chemical composition of the membranes of an insect virus and their relation to the virus and elementary bodies, J Bact 67, 210-216

Bertani, G, 1958, Lysogeny, in Advances in Virus Research, vol 5, pp 151-193, New York, Acad Press

Bertani, L E, 1957b, The effect of the inhibition of protein synthesis on the establishment of lysogeny, Virology 4, 53-71

Beveridge, W I B, and Burnet, F M, 1946, The cultivation of viruses and rickettsiae in the chick-embryo Med Res Counc Spec Report, Ser No 256, London

Blank, H, 1946, Radioactive isotopes for the study of animal viruses, Tr New York Acad Sc, ser 2, 18, 243-248

Bloch, D P, and Godman, G C, 1957, A cytological and cytochemical investigation of the development of the viral papilloma of human skin, J Exper Med 105, 161-176

Bloch, D P, Morgan, C, Godman, G C, Howe, C, and Rose, H M, 1957, A correlated histochemical and electron microscopic study of the intranuclear crystalline aggregates of adenovirus (RI-APC virus) in HeLa cells J Biophys Biochem Cytology 3, 1-8

Bodian, D, and Mellors, R C, 1945, The regenerative cycle of motoneurons, with special reference to phosphatase activity, J Exper Med 81, 469-488

———, 1947, Decrease of phosphocreatine in regenerating neurons, J Biol. Chem 167, 655-661

Bolton, E, 1954 Biosynthesis of nucleic acid in Escherichia coli, Proc Nat Acad Sc 40, 764-772

Bovarnick, M R, 1956, Phosphorylation accompanying the oxidation of glutamate by the Madrid E strain of typhus rickettsiae, J Biol Chem 220, 353-361

Boyer, G S, Leuchtenberger, C, and Ginsberg, H, 1957, Cytological and cytochemical studies of HeLa cells infected with adenoviruses, J Exper Med 105, 195-216

Breitenfeld, P M, and Schafer, W, 1957 The formation of fowl plague virus antigens in infected cells, as studied with fluorescent antibodies, Virology 4, 328-345

Brenner, S, 1957 Genetic control and phenotypic mixing of the adsorption cofactor requirement in bacteriophages T_2 and T_4, Virology 3, 560 574

Burton, K, 1955, The relation between the synthesis of deoxyribonucleic acid and the synthesis of protein in the multiplication of bacteriophage T_2, Biochem. J 61, 473-483

Hershey, A D, 1955, *An upper limit to the protein content of the germinal substance of bacteriophage* T_2, Virology *1*, 108-127.

Hershey, A D, and Burgi, E, 1956, Genetic significance of the transfer of nucleic acid from parental to offspring phage, Cold Spring Harbor Symp Quant Biol *21*, 91-101

Hershey, A D, and Chase, M, 1952, Independent functions of viral protein and nucleic acid in growth of bacteriophage J Gen Physiol *36*, 39-56

Hershey, A D, Dixon, J, and Chase, M, 1953, Nucleic acid economy in bacteria infected with bacteriophage T_2 I Purine and pyrimidine composition J Gen Physiol *36*, 777-789

Hershey, A D, Garen, A, Fraser, D K, and Hudis, J D, 1954, Growth and inheritance in bacteriophage, Carnegie Inst. of Washington Yrbk, No 53, 210-225

Hershey, A D, and Melechen, N E, 1957, Synthesis of phage-precursor nucleic acid in the presence of chloramphenicol, virology *3*, 207-236

Hollman, F G, Van den Ende, M, Kaplan, C, and Macpherson, C R, 1952, The effect of bacteriophage on the respiration of *Escherichia coli* and *Ps aeruginosa* South African J Clin Sc *3*, 66-76

Hook, A E, Beard, D, Taylor, A R, Sharp D G, and Beard, J W, 1946, Isolation and characterization of the T_2 bacteriophage of *Escherichia coli*, J Biol Chem *165*, 241-258

Howe, H A, and Bodian D, 1941, Refractoriness of nerve cells to poliomyelitis virus after interruption of the axons Bull Johns Hopkins Hosp *69*, 92-123

Howe, H A, and Flexner, J B, 1947, Succinic dehydrogenase in regenerating neurons J Biol Chem *167*, 663-671

Howe, H A, and Mellors, R C, 1945, Cytochrome oxidase in normal and regenerating neurons, J Exper Med *81*, 489 500

Hoyle, L, 1952, Structure of the influenza virus The relation between biological activity and chemical structure of virus fractions J Hyg *50*, 229-245

Hoyle, L, and Finter, N B, 1957, The use of influenza virus labelled with radiosulfur in studies of the early stages of the interaction of virus with the hostcell J Hyg *55*, 290-297

Hoyle, L, and Frisch-Niggemeyer, W, 1955, The disintegration of influenza virus particles on entry into the hostcell Studies with virus labelled with radiophosphorus J Hyg *53*, 474-486

Hoyle, L, Jolles, B, and Mitchell, R G, 1954, The incorporation of radioactive phosphorus in the influenza virus and its distribution in serologically active virus fractions, J Hyg *52*, 119-127

Huttin, T, Gustafsson, K, and Wessel, G, 1954, Incorporation of labeled metabolites in chorioallantoic membranes infected with influenza virus, Exp Cell Research *7*, 345-350

Hyden, H, 1947, The nucleoproteins in virus reproduction, Cold Spring Harbor Symp Quant Biol *12*, 104-114

Jacob, F, 1955, *Influence du régime carbone sur le developpement des bacteriophage*, Ann Inst Past *84*, 254-265

———, 1954, Mutation d'un bacteriophage induite par l'irradiation des seules bacteries-hotes avant l'infection, Comp Rend Acad Sc *233*, 732-734

Jeener, R, 1957, Biological effects of the incorporation of thiouracil into the ribonucleic acid of tobacco mosaic virus, Biochim et biophys acta *23*, 351-361

Jerne, N K, and Maaloe, O, 1957, The effects of ribonuclease on the bacteriophage T_4, its hostcell, *Escherichia coli* B, and on infected coli cells, Acta path et microbiol scandinav *40*, 362-368

Jesaitis, M A, 1957, The nucleic acids of T_2, T_4, and T_6 bacteriophages, J Gen Physiol *106*, 233-246

Karp, A, 1954, An immunological purification of typhus rickettsiae, J Bact *67*, 450-455

Kleczkowski J, and Kleczkowski, A, 1954, The effect of ribonuclease on phage-host interaction, J Gen Microbiol *11*, 451-458

Kleinschmidt, W J, Holmes, D H, and Behrens, O K, 1956, Vitamin content of purified typhus rickettsiae, tobacco mosaic virus, and coliphage T_6, Biochim et biophys acta *22*, 278-283

Knight, C A, 1946, Precipitin reactions of highly purified influenza viruses and related materials, J Exper Med *83*, 281-294

Koch, G, and Weidel, W, 1956, Abspaltung chemischer komponenten of Colimembran durch daran adsorbierte phagen, Ztschr Naturforsch, *11b*, 345-355

Kovacs, E, 1956a Comparative biochemical studies on normal and poliomyelitis infected tissue cultures IV Enzyme-changes in host cells Proc Soc Exper Biol & Med *92*, 183 188

———, 1956b, Comparative biochemical studies on normal and on poliomyelitis virus-infected tissue cultures V Profound alteration of acid and alkaline phosphatase activity in infected Rhesus kidney cells J Exper Med *104*, 589-613

Kozloff, L M, 1952a, Biochemical studies of virus reproduction VI The breakdown of bacteriophage T_4r^+ J Biol Chem *194*, 83-93

———, 1952b, Biochemical studies of virus reproduction VII The appearance of parent nitrogen and phosphorus in the progeny J Biol Chem *194*, 95-108

Kozloff, L M, 1953, Origin and fate of bacteriophage material, Cold Spring Harbor Symp Quant Biol *18*, 209-220

Kunkee, R E, and Pardee, A B, 1956, Studies in the role of deoxyribonuclease in T_2 bacteriophage development, Biochim et biophys acta *19*, 236-246

Labaw, L W, 1951 The origin of phosphorus in *Escherichia coli* bacteriophages, J Bact *62*, 169-173

Labaw, L W, 1953, The origin of phosphorus in the T_1, T_3, T_6, and T_7 bacteriophages of *Escherichia coli*, J Bact *66*, 429-436

Lanni, F, and Lanni, Y T, 1953, Antigenic structure of bacteriophage, Cold Spring Harbor Symp Quant. Biol *18*, 159-168

virus by the HeLa cell, J Exper Med *104*, 271-287

Eagle, H, Piez, K A, and Fleischman, R, 1957, The utilization of phenylalanine and tyrosine for protein synthesis by human cells in tissue culture, J Biol Chem *228*, 847-862

Eaton, M D, 1952, Observations on growth of virus and the energy-yielding activities of the host cell, Arch ges Virusforschung *5*, 53-72

Eckert, E A, Green, I, Sharp, D G, Beard, D, and Beard, J W, 1955b, Virus of avian erythromyeloblastic leukosis VII Thermal stability of virus infectivity, of the virus particle, and of the enzyme dephosphorylating adenosine triphosphate J Nat Cancer Inst *16*, 153-161

Eckert, E A, Sharp, D G, Beard, D, Green, I, and Beard, J W, 1955a, Virus of avian erythromyeloblastic leukosis IX Antigenic constitution and immunologic characterization J Nat Cancer Inst *16*, 593-644

van den Ende, M, 1950, The utilization of amino acid solutions by virus-infected eggs, studied by paper chromatography, J Gen Microbiol *4*, 277-279

Fernandez, B, Haas, F. L and Wyss. O, 1953, *Induced host-range mutations in bacteriophage*, Proc Nat Acad Sc *39*, 1052-1057

Fisher, T N, and Ginsberg, H S, 1956, The reaction of influenza viruses with guinea pig polymorphonuclear leucocytes II The reduction of white blood cell glycolysis by influenza viruses and receptor destroying enzyme (RDE) Virology *2*, 637-655

Flaks, J G, and Cohen, S S, 1957, The enzymic synthesis of 5-hydroxymethyldeoxycytidylic acid, Biochim et biophys acta *25*, 667-668

———, 1958, The deoxycytidylate hydroxymethylase of virus infected *Escherichia coli*, Fed Proc *17*, 220

Foster, R A C, 1948, An analysis of the action of proflavine on bacteriophage growth, J Bact *56*, 795-809

Fowler, C B, 1950, La fermentation anaerobie de *Escherichia coli* consideree dans ses rapports avec l'adaptation enzymatique, Comp, rend Acad Sc *231*, 1348-1350

Fowler, C B, and Cohen, S S. 1948, Chemical studies in host-virus interactions IV A method of determining nutritional requirements for bacterial virus multiplication J Exper Med *87*, 259-274

Fox, M S, 1957, *Deoxyribonucleic acid incorporation* by transformed bacteria, Biochim et biophys acta *26*, 83-85

Franklin, R M, Rubin, H., and Davis, C A, 1957, The production, purification, and properties of Newcastle disease virus labeled with radiophosphorus, Virology *3*, 96-114.

Fraser, D, and Mahler, H R, 1957, The effects of nucleases on the reproduction of T_4 bacteriophage in protoplasts of *Escherichia coli*, Arch Biochem *69*, 166-177

Fraser, D, Mahler, H R, Shug, A L, and Thomas, C A, 1957, The infection of sub-cellular *Escherichia coli, strain B, with free DNA from* T_2 bacteriophage, Proc Nat Acad Sc, in press

French, R C, 1954, The contribution from parent to progeny in T_2 coliphage, J. Bact *67*, 45-49

French, R C., Graham, A F, Lesley, S M and van Rooyen, C. E, 1952 The contribution of phosphorus from T_2r+ bacteriophage to progeny, J Bact *64*, 597-607

French, R C, and Siminovitch, L, 1955, The action of T_2 bacteriophage ghosts on *Escherichia coli* B, Canad J Microbiol *1*, 757-774

Friedman, S, 1953, The purine and pyrimidine metabolism of normal and phage-infected *Escherichia coli*, J Biol Chem *201*, 125-135

Gaylord, W H, and Melnick, J L, 1953, Developmental forms of vaccinia virus, Science *117*, 10-13

Gersh, I, and Bodian, D, 1943, Some chemical mechanisms in chromatolysis, J Cell & Comp Physiol *21*, 253-279

Gifford, G E, and Syverton, J T, 1957, Replication of poliovirus in primate cell cultures maintained under anaerobic conditions, Virology *4*, 216-223

Gladstone, V F C, 1942, Study of respiration in healthy and mosaic-infected tobacco plants, Plant Physiol *17*, 267-277

Goodgal, S H, 1956, Incorporation of phage DNA on lysogenization of Shigella dysenteriae, Biochim et biophys acta *19*, 333-340

Gottshalk, A, 1957, Virus enzymes and virus templates, Physiol Rev *37*, 66-83

Graham, A F, 1950, The chemical analysis of purified influenza virus A (PR8 strain) containing radioactive phosphorus, Canad J Res, Sect *E 28*, 186-195

———, 1953, The fate of the infecting phage particle, Ann Inst Past *84*, 90-99

Gratia, A, Brachet, J, and Jeener, R, 1945, Etude histochimique et microchimique des acides nucleiques au cours de la grasserie du ver a soie, Bull Acad roy méd Belgique *10*, 72-81

Hamers-Casterman, H, and Jeener, R, 1957, An initial ribonuclease-sensitive phase in the multiplication of tobacco mosaic virus, Virology *3*, 197-206

Heagy, F C, 1950, The effect of 2,4 dinitrophenol and phage T_2 on *Escherichia coli* B, J Bact *59*, 367-373

Heggie, A D, and Morgan, H R, 1956, Latent viral infection of cells in tissue culture III Role of certain amino acids Proc Soc Exper Biol & Med *92*, 506-509.

Henle, W, 1953, Developmental cycle in animal viruses, Cold Spring Harbor Symp Quant Biol *18*, 35-44

———, 1956, The use of phosphorus-32 in the study of incomplete forms of influenza virus, Tr. New York Acad Sc ser *2*, *18*, 255-260

Herriott, R M, 1951, Nucleic-acid free T_2 virus "ghosts" with specific biological action, J Bact. *61*, 752-754

Hershey, A. D, 1952, Nucleic acid economy in bacteria infected with bacteriophage T_2 II Phage precursor nucleic acid J Gen Physiol *37*, 1-23.

Morgan, C., Howe, C., Rose, H. M., and Moore, D. H., 1956, Structure and development of viruses observed in the electron microscope IV Viruses of the RI-APC group J Biophys Biochem Cytology 2, 351-360

Moulder, J. W., McCormack, B. R. S., Itatani, M. K., 1953, Energy requirements for synthesis of feline pneumonitis virus in isolated chick embryo yolk sac, J Infect Dis 93, 140-149

Moulder, J. W., and Weiss, E., 1951, Respiratory metabolism of the extra-embryonic membranes of chick embryos in relation to multiplication of the agent of feline pneumonitis, J Infect Dis 89, 65-76

Nickell, L. G., and Brakke, M. K., 1954 Secretion of α-amylase by Rumex virus tumors in vitro Biological studies Am J Botany 41, 390-394

Novick, A., and Szilard, L., 1951 Virus strains of identical phenotype but different genotype, Science 113, 34-35

Overman, J. R., and Tamm, I., 1957, Multiplication of vaccinia virus in the chorio-allantoic membrane in vitro, Virology 3, 173-184

Pardee, A. B., and Kunkee, R. E., 1952, Enzyme activity and bacteriophage infection II Activities before and after infection J Biol Chem 199, 9-24

Pardee, A. B., and Williams I., 1952, The increase in deoxyribonuclease in virus infected Escherichia coli, Arch Biochem 40, 222-223

Park, B. H., 1956, An enzyme produced by a phage-host cell system I The properties of a Klebsiella phage Virology 2, 711-718

Pearson, H. E., and Winzler R. J. 1949 Oxidative and glycolytic metabolism of minced day-old mouse brain in relation to propagation of Theiler's GD VII virus, J Biol Chem 181, 577-582

Puck, T. T., and Fisher H. W., 1956 Genetics of somatic mammalian cells I Demonstration of the existence of mutants with different growth requirements in a human cancer cell strain (HeLa) J Exper Med 104, 427-434

Puck, T. T., and Lee, H. H., 1954 Mechanism of cell wall penetration by viruses I An increase in host cell permeability induced by bacteriophage infection J Exper Med 99, 481-494

———, 1955, Mechanism of cell wall penetration by viruses II Demonstration of cyclic permeability change accompanying virus infection of Escherichia coli B cells J Exper Med 101, 151-175

Puck, T. T., and Marcus, P. I., 1956, Action of x-rays on mammalian cells, J Exper Med 103, 653-666

Puck, T. T., Marcus, P. I., and Cieciura, S. J., 1956, Clonal growth of mammalian cells in vitro, J Exper Med 103, 273-284

Putnam, F. W., and Kozloff, L. M., 1950, Biochemical studies of virus multiplication IV The fate of the infecting virus particle J Biol Chem 182, 243-250

Putnam, F. W., Miller, D., Palm, L., and Evans, E. A., 1952, Biochemical studies of virus reproduction X Precursors of bacteriophage T_7 J Biol Chem 199, 177-191

Racker, E. (ed.), 1954, Metabolism of infected cells, in Cellular Metabolism and Infections, Symposium 8, p 138, New York Acad Med New York, Acad Press

Racker, E., and Kabat, H., 1942, The metabolism of the central nervous system in experimental poliomyelitis, J Exper Med 76, 579-585

Rafelson, M. E., Winzler, R. J., and Pearson, H. E., 1949, The effects of Theiler's GD VII virus on P^{32} uptake by minced one day-old mouse brain, J Biol Chem 181, 583-591

Reddi K. K., 1956, Isolation and properties of ribosenucleic acid from tobacco leaves, Biochim et biophys acta 24, 209-210

Roberts, R. B., Abelson, P. H., Cowie, D. B., Bolton, E. T., and Britten R. J. 1955, Studies in biosynthesis in Escherichia coli Carnegie Inst Washington Pub., No 607

Rubin, H., Baluda, M., and Hotchin, J. E., 1955 The maturation of western equine encephalomyelitis virus and its release from chicken embryo cells in suspension, J Exper Med 101, 205-212

Rubin, H., and Franklin R. M., 1957, On the mechanism of Newcastle disease virus neutralization by immune serum, Virology 3, 84-95

Schafer, W., 1957, Units isolated after splitting fowl plague virus in The Nature of Viruses, Ciba Foundation Symposium, pp 91-103 London Churchill

Scherer, W. F., 1952, Agglutination of a pure strain of mammalian cells (L Strain, Earle) by suspensions of vaccinia virus Proc Soc Exper Biol & Med 80, 598-602

Schlesinger, M., 1934 Zur Frage der Chemischen Zusammensetzung des Bakteriophagen Biochem Ztschr 273, 306-311

———, 1936, The feulgen reaction of the bacteriophage substance Nature, London 138, 508-509

Scott, D. B. M., 1956, The oxidative pathway of carbohydrate metabolism in Escherichia coli 3 Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in cells grown under different conditions Biochem J 63, 587-593

Scott, D. B. M., and Cohen S. S. 1953, The oxidative pathway of carbohydrate metabolism in Escherichia coli 1 Quantitative studies of glucose 6-phosphate dehydrogenase and 6 phosphogluconate dehydrogenase Biochem J 55, 33-36

Sellers, M. I., and Jann, G. J. 1954, Biochemical changes in mouse lung concomitant with influenza virus infection, Proc Soc Exper Biol & Med 86, 205-212

Shaw, M., and Samborski, D. J., 1957 The physiology of host-parasite relations III The pattern of respiration in rusted and mildewed cereal leaves, Canad J Bot 35, 389-407

Siegel, A., Ginoza, W., and Wildman, S. G., 1957, The early events of infection with tobacco mosaic virus nucleic acid Virology 3, 554-559

Siegel, A., Singer S. J., and Wildman, S. G., 1952, A preliminary study of the high-molecular weight compounds of normal and virus-infected Escherichia coli, Arch Biochem 41, 278-293

Siegel, A., Wildman, S. G., and Ginoza, W., 1956, Sensitivity to ultraviolet light of infectious tobacco

Lanni, Y T, 1954, Infection by bacteriophage T_5 and its intracellular growth—a study by complement fixation, *J Bact 67*, 640-650

Lanning, M C, and Cohen, S S, 1955, On the origin of deoxyribose in *Escherichia coli* and T_6r+ bacteriophage, *J Biol Chem 216*, 413-423

Latarjet, R, 1948, Intracellular growth of bacteriophage studied by roentgen irradiation, *J Gen Physiol 31*, 529-546

Le Clerc, J, 1956, Action of ribonuclease on the multiplication of the influenza virus, Nature, London *177*, 578-579

Lerman, L S, and Tolmach, L J, 1957, Genetic transformation I Cellular incorporation of DNA accompanying transformation in Pneumococcus Biochim et biophys acta, *26*, 68-82

Lesley, S M, French, R C, Graham, A F, and van Rooyen, C E, 1951, Studies on the relationship between virus and host cell II The breakdown of T_2r+ bacteriophage upon infection of its host *Escherichia coli* Canad J M Sc *29*, 128-143

Lesley, S M, and Graham, A F, 1956, The utilization of desoxyribonucleotide phosphorus by *Escherichia coli*, Canad J Microbiol *2*, 17-27

Levine, A S, Bond, P H, Scala, A R, and Eaton, M D, 1956 Studies on the relationship of potassium to influenza virus multiplication, J Immunol *76*, 386-392

Levinthal, C, 1956, The mechanism of DNA replication and genetic recombination in phage, Proc Nat Acad Sc *42*, 394-404

Levinthal, C, and Fisher, H, 1952, The structure development of a bacterial virus, Biochim et biophys acta *9*, 419-429

Levinthal, C, and Thomas, C A, 1957a, Molecular autoradiography the β-ray counting from single virus particles and DNA molecules in nuclear emulsions Biochim et biophys acta *23*, 453-465

———, 1957b, The molecular basis of genetic recombination in phage, *in* Chemical Basis of Heredity, McElroy, W, and Glass, B (eds), pp 735-743, Baltimore, Johns Hopkins Press

Levy, H B, and Baron, S, 1957, The effect of animal viruses on host cell metabolism II Effect of poliomyelitis virus on glycolysis and uptake of glycine by monkey kidney tissue cultures J Infect. Dis *100*, 109-118

Leyon, H, 1953, Virus formation in chloroplasts, Exp Cell Res *4*, 362-370

Litman, R M, and Pardee, A [redacted], 1956, Production of bacteriophage mutants by a disturbance of deoxyribonucleic acid metabolism, Nature, London *178*, 529-532

Liu, O C, 1956, The labeling of influenza virus with radioactive phosphorus, Tr New York Acad Sc, ser. 2, *18*, 249-254

Luria, S E, 1947, Reactivation of irradiated bacteriophage by transfer of self-reproducing units, Proc Nat Acad Sc. *33*, 253-264

———, 1951, The frequency distribution of spontaneous bacteriophage mutants as evidence for the exponential rate of phage reproduction, Cold Spring Harbor Symp Quant Biol *16*, 463-470

———, 1953, Host-induced modifications of viruses, Cold Spring Harbor Symp Quant Biol *18*, 237-244

Luria, S E, Delbruck, M., and Anderson, T F., 1943, Electron microscope studies of bacterial viruses, J Bact *46*, 57-78

Luria, S E, and Human, M. L, 1950, Chromatin staining of bacteria during bacteriophage infection, J Bact *59*, 551-560.

Luria, S E, and Latarjet, R., 1947, Ultraviolet irradiation of bacteriophage during intracellular growth, J Bact *53*, 149-163.

Maaløe, O, and Symonds, N, 1953, Radioactive sulfur tracer studies on the reproduction of T_4 bacteriophage, J Bact *65*, 177-182

Maaløe, O, and Watson, J D, 1951, The transfer of radioactive phosphorus from parental to progeny phage, Proc Nat Acad Sc *37*, 507-513

McLimans, W F, Siem, R A, Mark, [redacted] C, and Pinska, E, 1950, A physiological study of virus parasitism II The effect of environmental temperature on the rates of oxygen consumption of normal eggs and eggs infected with Newcastle Disease virus J Immunol *64*, 475-485

Marcus, P I, Cieciura, S J, and Puck, T T, 1956, Clonal growth in vitro of epithelial cells from normal human tissues, J Exper Med *104*, 615-628

Matthews, R [redacted] F, and Smith, J D, 1955, The chemotherapy of viruses, *in* Advances in Virus Research, vol 3, pp 49-148, New York, Acad Press

Meneghini, M, and Delwiche, C C, 1951, The multiplication of tobacco mosaic virus in the host tobacco plant, J Biol Chem *189*, 177-186

Miller, E M, and Goebel, W F, 1954, The nature of prophage in lysogenic *Bacillus megatherium*, J Exper Med *100*, 525-540

Miroff, G, Cornatzer, W E, and Fischer, R G, 1957, The effect of poliomyelitis virus type 1 (Mahoney strain) on the phosphorus metabolism of the HeLa cell, J Biol Chem *228*, 255-262

Mogabgab, W J, and Horsfall, F L, 1952, Effect of sodiummonofluoroacetate on the multiplication of influenza viruses, mumps virus, and pneumonia virus of mice (PVM), J Exper Med *96*, 531 548

Moldave, K, 1954, The effect of Theiler's G D VII virus on the intracellular distribution of radiophosphorus in mouse brain in vitro J Biol Chem *210*, 343-345

Mommaerts, [redacted] B, Sharp, D G, Eckert, E A, Beard, D, and Beard, J W, 1954 Virus of avian erythromyeloblastic leukosis I Relation of specific plasma particles to the dephosphorylation of adenosine triphosphate J Nat Cancer Inst *14*, 1011-1025

Monod, J, and Wollman, [redacted], 1947, L'inhibition de la croissance et de l'adaptation enzymatique chez les bactéries infectées par le bactériophage, Ann Inst Pasteur *73*, 937-957

Morgan, C Ellison, S A, Rose, H M, and Moore, D H, 1954, Structure and development of viruses observed in the electron microscope II Vaccinia and fowl pox viruses J. Exper Med *100*, 301-310

Wolf, H L., 1957, Changes in desoxyribonucleic acid and in glycoprotein content of mouse-lung epithelial cells during influenza virus infection, J Path & Bact 73, 337-346

Wood, H G, 1944, Metabolism of nervous tissue in poliomyelitis, Journal-Lancet 64, 240-242

———, 1955, Significance of alternate pathways [illegible] the metabolism of glucose, Physiol Rev 35, 841-859

Wyatt, [illegible] R, and Cohen, S S, 1953, The bases of the nucleic acids of some bacterial and animal viruses: the occurrence of 5-hydroxymethylcytosine, Biochem J 55, 774-782

Wyatt, G R, Loughheed, T C., and Wyatt, S S, 1956, The chemistry of insect hemolymph, J Gen. Physiol 39, 853-868

Wyatt, S S, 1956, Culture in vitro of tissue from the silkworm, Bombyx mori L. J Gen Physiol 39, 841-852

Wynd, F L, 1942, Certain enzymatic activities of normal and mosaic infected tobacco plants, J Gen Physiol 25, 649-661.

———, 1943, Metabolic phenomena associated with virus infection in plants, Botanical Rev 9, 395-465

Zeros, N, 1956, The virogenic stroma in nuclear and cytoplasmic polyhedroses, Nature, London 178, 412-413

mosaic virus nucleic acid, Nature, London *178*, 1117-1118

Siminovitch, L, 1953, Biochemical modifications of the bacterial host during bacteriophage development, Ann Inst Pasteur *84*, 265-273

Siminovitch, L, and Graham, A F, 1955, Synthesis of nucleic acids in *Escherichia coli*, Canad J Microbiol. *1*, 721-732

———, *1956*, *Significance of ribonucleic acid and* deoxyribonucleic acid turnover studies, J Histochem *4*, 508-515

Siminovitch, L, Graham, A F, Lesley, S M, and Nevill, A, 1957, Propagation of L strain mouse cells in suspension, Exp Cell Res *12*, 299-308

Sinsheimer, R L, 1954, Nucleotides from T_2r+ bacteriophage, Science *120*, 551-553

———, 1956, The glucose content of the deoxyribonucleic acids of certain bacteriophages, Proc. Nat Acad Sc *42*, 502-504

Smadel, J E, and Hoagland, C L, 1942, Elementary bodies of vaccinia, Bact Rev *6*, 79-110

Smith, M H D, and Kun, E, 1954, Morphological and biochemical studies on the chorioallantois of the chick embryo following infection with certain viruses, Brit J Exper Path *35*, 1-10

Spizizen, J, 1957a, Infection of protoplasts by disrupted T_2 virus, Proc Nat Acad Sc *43*, 694-701

———, 1957b, The effect of virus infection on pyruvate metabolism, Biochim et biophys acta *23*, 333-341

Sprince, H, and Schoenbach, E B, 1942, An analysis of tobacco mosaic virus for biotin, riboflavin, and pantothenic acid, Proc Soc Exper Biol & Med *49*, 415-418

Stent, G S, 1955, Decay of incorporated radioactive phosphorus during reproduction of bacteriophage T_2, J Gen Physiol *38*, 853-865

Stent, G S, and Fuerst, C R, 1956, Decay of incorporated radioactive phosphorus during development of a temperate bacteriophage, Virology *2*, 737-752

*Stent, G. S, and Maaløe, O, 1953, Radioactive phos*phorus tracer studies on the reproduction of T_4 bacteriophage II Kinetics of phosphorus assimilation Biochim et biophys acta *10*, 55-69

Stewart, D C, and Kirk, P L, 1954, The liquid medium in tissue culture, Biol Rev *29*, 119-153

Streisinger, G, and Weigle, J, 1956, Properties of bacteriophages T_2 and T_4 with unusual inheritance, Proc Nat Acad Sc *42*, 504-510

Tamm, I, *1956*, Selective chemical inhibition of influenza virus, J Bact. *72*, 42-53

Tamm, I, Folkers, K, and Shunk, C H, 1956, High inhibitory activity *of* certain halogenated ribo furanosylbenzimidazoles in influenza V virus multiplication, J Bact *72*, 54-58

Tamm, I, and Overman, J R, 1956, Relationship between structure of benzimidazole derivatives and inhibitory *activity on vaccinia virus multiplication*, Virology *3*, 185-196

Tanami, Y, 1957, Frequency distribution of host range mutants among T_2 phage infected bacteria, J Bact *73*, 513-515

Tenenbaum, E, 1957, Changes in cellular nucleic acids during infection with poliomyelitis virus as studied by fluorescence microscopy, Nature, London *180*, 1044

Thompson, R L, 1947, The effect of metabolites, metabolite antagonists and enzyme-inhibitors on the growth of the vaccinia virus in Maitland type tissue cultures, J Immunol *55*, 345-352.

Tomizawa, J, and Sunakawa, S, 1956, The effect of chloramphenicol on deoxyribonucleic acid synthesis and the development of resistance to ultraviolet irradiation in *Escherichia coli* infected with bacteriophage T_2, J Gen Physiol *39*, 553-565

Utter, M F, Reiner, J M, and Wood, H G, 1945, Measurement of anaerobic glycolysis in brain as related to poliomyelitis, J Exper Med *82*, 217-226

Vidaver, G, and Kozloff, L M, 1957, The rate of synthesis of deoxyribonucleic acid in *Escherichia coli* B infected with T_2r+ bacteriophage, J Biol Chem *225*, 335-347

Volkin, E, 1954, The linkage of glucose in coliphage nucleic acids, J Am Chem Soc *76*, 5892

Volkin, E, and Astrachan, L, 1957, RNA metabolism in T_2-infected *Escherichia coli*, *in* The Chemical Basis of Heredity, McElroy, W, and Glass, B (eds), pp 686-695, Baltimore, Johns Hopkins Press

Wahl, R, Lee, K Y, and Barbu, E, 1957, Concentration, purification et analyse de trois bacteriophages, Ann Inst Pasteur *92*, 153-165

Watanabe, I, 1957, The effect of ultraviolet light on the production of bacterial virus protein, J Gen Physiol *40*, 521-531

Watanabe, I, Stent, G S, and Schachman, H K, 1954, On the state of the parental phosphorus during reproduction of bacteriophage T_2, Biochim et biophys acta *15*, 38-49

Watson, B K, and Coons, A H, 1954, Studies of influenza virus infection in the chick embryo using fluorescent antibodies, J Exper Med *99*, 419-428

*Watson, J D, and Crick, F H C, 1953, The struc*ture of DNA, Cold Spring Harbor Symp Quant Biol *18*, 123-131

Watson, J D, and Maaløe, O, 1953, Nucleic acid transfer from parental to progeny bacteriophage, Biochim et biophys acta *10*, 432-442

Wecker, E, and Schafer, W, 1956, Einbau von radioaktivem Phosphor in das virus des klassischen Geflugelpest, Ztschr Naturforsch *11b*, 181-187

Weed, L L, and Cohen, S S, 1951, The utilization of host pyrimidines in the synthesis of bacterial viruses, J Biol Chem *192*, 693-700

Weigle, J J, 1953, Induction of mutations in a bacterial virus, Proc Nat Acad Sc *39*, 628-636

Weigle, J J, and Dulbecco, R, 1953, Induction of mutations in bacteriophage T_3 by ultraviolet light, Experientia *19*, 372

*Wielgosz, G S, 1957, Pyruvate metabolism of chorio*allantoic membrane infected with influenza A, Virology *3*, 475-484

Williams, R C, and Smith, K M, 1957, A crystallizable insect virus, Nature, London *179*, 119-120

portance, and the main motivation of work in this field was based on the need for control of these infections In a similar way, the work on animal viruses was closely linked to medical and veterinary practice

Animal virology grew up largely as an offshoot of pathogenic bacteriology and, in the beginning, was largely in the hands of pathologists Both medical and veterinary virologists were mainly interested in the broad, general aspects of disease, the mode of virus transmission, the insect vector, methods of virus isolation and antibody measurement as aids to laboratory diagnosis, and the study of the development and the duration of systemic immunity. Apart from studies on inclusion bodies, relatively little emphasis was placed on the basic biologic unit, the virus-infected cell. The reasons for this neglect were in part technical, the difficulties of carrying out virus studies in tissue culture and of cultivating pure lines of mammalian cells But a more important factor was the immediate enormous practical success which followed the general approach A good example is urban yellow fever, which was completely controlled through the limited knowledge that the filterable agent circulated in the blood of an infected man and was transmissible to other men by mosquitoes On the other hand the study of the virus-infected cell, admittedly a recent approach, has had very little direct impact on man's ability to control virus infections of either plants or animals

It is at the level of cellular infection that the unity of the several branches of virus research is demonstrated most readily, and the most important single event in the development of virology as an independent science was the discovery of bacterial viruses (Twort, 1915, d'Herelle, 1917) Early development of this field was slow Efforts were dissipated over a wide variety of available agents A number of attempts were made to use bacteriophage therapeutically in human bacterial infections, but the results were disappointing and, when interest in bacteriophage was renewed in the late 1930 s, investigators were not looking for immediate medical or economic consequences, the primary interest was biologic Virus studies were focused consistently, for the first time, at the level of the infected cell, and the results were exceedingly fruitful Bacterial virology has become of enormous importance in various fields of biology, in cytology, biochemistry, bacterial genetics, general genetics and animal virology

The effect of this development on animal virology has been especially marked. Studies on phage multiplication, on the eclipse phase, on genetic constitution, on multiplicity reactivation and cross-reactivation and on phenotypic mixing have served as models for studies with animal viruses Even phage technics, such as plaque formation, for particle enumeration have been applied directly to animal viruses and have been carried into this field by students of bacteriophage Concomitant with this development has been an increased interest in the study of isolated mammalian cells, and it is tempting to speculate that the study of cells in tissue culture will eventually complement virologic studies just as bacterial and bacteriophage studies have become interdependent

The study of plant viruses presents both outstanding advantages and disadvantages for solving virus problems Plant viruses are relatively simple in structure, they can be obtained readily in great quantity and are easily purified by chemical methods. On the other hand, plant virus particles infect with exceedingly low efficiency, they are difficult to titrate with accuracy, and so far infection has not been studied successfully at the cellular level Plant viruses have proved to be useful models Interference between viruses and infection of cells with naked RNA was accomplished first with plant viruses and later with other agents.

In the ensuing account bacterial animal plant and insect infections with viruses are described separately. For each group a model virus is used the "T" viruses in the case of bacteriophage, the myxo group (influenza mumps, Newcastle disease and fowl plague) for animal viruses, and tobacco mosaic as a representative of plant viruses In each case, the description of these particular agents will form the backbone of the discussion of multiplication Material with other viruses will be used to illustrate special points The distribution of the available experimental evidence almost forces this stratagem, and the justification of generalizing from these models will

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4

Virus-Host Cell Relation

INTRODUCTION *

Virology is a new science with origins in widely scattered and independent developments involving plant, animal, bacterial and insect infections The early emphasis in the study of these varied agents was on the *systemic* effects that they produced. This tended to promote the separate development of the various branches since, in terms of symptoms, it is difficult to discern much connection between yellow fever and mosaic disease of tobacco, for example, although both are caused by small subcellular particles The existence of general principles in virology has become apparent only in recent years since virus infections have been studied with increasing success at the cellular level, and the development of a virologic science is still in a fairly primitive stage While there are numerous gaps in our knowledge which dictate the use of restraint in formulating new generalities, the recent impact of one branch of the field on another has been so great and of such importance that no one can any longer doubt the heuristic as well as the didactic value of the comparative approach to this science.†

Extracellular virus particles, having no intrinsic metabolism, are in a relatively stable state of suspended animation which permits an accurate description of their chemical and physical properties However, during most of their *intracellular* life, viruses are undergoing constant change, and they become transformed so markedly that they are no longer recognizable by conventional methods Virus adsorption is followed by virus penetration, and the stable infective form is transformed into a vegetative noninfective stage, after which there is replication and biosynthesis of individual virus components, assembly of these components in a process called "maturation" and, finally, the extrusion of new virus from the cell The study of these events constitutes the core of virology The aim of the author in this chapter will be to discuss viruses on a comparative basis and to confine the discussion, wherever possible, to infection at a cellular level The weakness of this approach is its prematurity, since the study of animal viruses in isolated cells is just beginning

The inception of virology (Iwanowski, 1892) coincided with the failure of a standard bacteriologic tool, the earthenware filter, to give a pathogen-free filtrate of the sap of tobacco plants which had been infected with mosaic disease virus In exploiting this finding, plant pathologists confined their attention primarily to plants of agricultural im-

"abo- ... afer ... isive formulation of virology as a science in his textbook *General Virology* (Luria, 1953) He has also published a subsequent review on *The Reproduction of Viruses,* (Luria, 1958) which was made available by the author in prepublication form, and this article, together with the textbook, have been freely used in this chapter as a model for both form and substance

in nature is temperate in character and is carried in lysogenic form. Many bacteria are lysogenized by more than one type of virus and the number of virus types is large, especially if minor differences are counted.

Among vertebrates, a few virus infections of fish are known (fishpox, salmon poisoning), and a tumor virus of the leopard frog is common. On the basis of experience with mammals, it is possible that virus infections of cold-blooded vertebrates may be common but undetected because of the lack of close observation. Among birds, virus infections are extremely common. In the domestic fowl they are responsible for a wide variety of pathologic conditions, including malignant tumors. Among mammals, it is a reasonable guess that none are free from infection with viruses, although the domestic and laboratory species furnish the largest number of established examples.

Among insects, viruses are known which infect hymenoptera, diptera and lepidoptera.

CLASSIFICATION OF VIRUSES

Numerous attempts have been made to classify all known viruses under one comprehensive system. No one has yet evolved a satisfactory solution to this problem, which may be partly due to the fact that so little is known concerning the origin and the evolution of viruses that the various schemes contain no continuous thread of natural significance. Attempts have been made to classify viruses on the basis of host range, size, morphology, type of lesion produced, the antigenic constitution and other criteria. Usually it is found that a single criterion (antigenic relationship, for example) works well with one group of agents but produces paradoxes in another group. Because of the very tentative character of most of these schemes, they will not be discussed in detail.

Animal virologists (Andrewes, 1954) have worked out a preliminary plan in which 5 small groups of animal viruses are described. Agents in these groups are similar morphologically and have at least one other feature in common. Only a small fraction of the known animal viruses fall within this classification.

1. *The Chlamydozoaceae* (mantle viruses). This includes psittacosis, ornithosis, trachoma, inclusion conjunctivitis and lymphogranuloma venereum viruses. These agents are of uniformly large size. They are morphologically complex and are all sensitive to antibiotics. In the opinion of some virologists, these agents are more closely related to the rickettsiae than to viruses and do not properly fall within the scope of this classification.

2. Pox viruses (Fenner and Burnet, 1957). This group includes the viruses of smallpox, vaccinia, cowpox, ectromelia (mousepox), fowlpox, molluscum contagiosum, myxoma and fibroma. They are all fairly large viruses, they have antigens in common, and all produce similar cytoplasmic inclusions. Cells infected with these agents show varying degrees of proliferative as well as necrotic reactions.

3. This group consists of herpes virus, B virus, pseudorabies and varicella. Like the pox viruses, they are capable of causing vesicles in the skin, and they have a tendency to infect the central nervous system.

4. Poliomyelitis viruses, types I, II and III, and mouse poliomyelitis (von Magnus et al., 1955). These are enteric viruses which spread occasionally to the central nervous system.

5. Myxoviruses (Andrewes et al., 1955). This group includes influenza A, B, C and D, fowl plague virus (FPV), Newcastle disease virus (NDV) and mumps. All of these agents possess an enzyme capable of splitting off neuraminic acid derivatives (Gottschalk, 1957) from some common mucoproteins.

SEROLOGIC PROPERTIES OF VIRUSES

All viruses are good antigens or, more precisely, they contain good antigens, and the virus-antibody reaction is of special interest for a variety of reasons. From the clinical and epidemiologic standpoint interest stems from the fact that, in higher organisms, the development of antibodies as a response to infection is closely correlated with the development of immunity. From the diagnostic standpoint, circulating antibodies are of great retrospective value in establishing the etiology of infections. However, the applied aspects of serology are covered in chapters concerned with specific virus diseases and with serologic reactions (Chap. 10).

The present chapter deals with the kinetics of virus-antibody reactions and with the use

have to be judged in the light of accumulating evidence on other agents.

DEFINITION OF A VIRUS

For reasons of clarity, it is desirable to try to define what is meant by a virus. Luria (1953) has defined viruses by introducing four basic restrictions (1) viruses are entities, (2) viruses are submicroscopic in size, (3) viruses reproduce only inside specific living cells, and (4) viruses can be introduced into these host cells from without

The notion that a virus is an entity with specific characteristics and specific structure is a fact which is made clear in both this and other chapters of this book Evidence regarding the microscopic structure is given in Chapter 2 These details of structure, plus the discovery of the manner in which genetic traits are carried and transmitted in viruses, have done more than anything else to dispel the once widely held notion that viruses are merely degraded or slightly altered normal cell constituents

Limitations on the size of viruses seems, at first, to be arbitrary, not to say somewhat questionable, since the total range is so very great Actually, the outmoded term "filterable viruses" arose from the fact that earthenware filters effected a fairly satisfactory separation between most viruses and most bacteria Viruses range in size from those which are of the approximate dimension of large protein molecules on up to those which are barely visible in the light microscope

The third requirement states that the agent, *in order to be a virus, must be able to multiply within specific living cells* The word "specific" implies a definite but limited range of suitable host cells The term "living" may be regarded as describing a state of metabolic activity or of ability to mobilize energy rather than capacity to divide, because the latter is often lost immediately after the virus enters the cell.

Some of the smallest bacteria (pleuropneumonialike organisms and micro-organisms found in sewage) meet the size requirements but are eliminated because they are not obligatory intracellular parasites On the other hand, certain bacteria and algae which multiply only within cells are large and are eliminated by the size requirement.

The fourth restriction, that the agent can be introduced from without, is a necessary one in order to exclude subcellular particles such as mitochondria or microsomes from the definition It is not impossible that, with increasing knowledge of the origin of viruses, this restriction will come to have less and less meaning.

The difficulties in definition are pointed up by the discovery that naked RNA without accompanying protein from plant viruses is able to infect plants What was once considered to be *the* virus appears to be only a stable vehicle containing the essential infective entity. A chemist, extracting DNA from pneumococci and using it to transform other pneumococci, is doing something operationally very similar to the infection of plant cells with naked RNA, yet common sense tells us that the transforming principle is not a virus. We have clearly reached some kind of borderline of differentiation Luria (1958) has suggested as a unifying view that viruses be considered as "genetically specific cell constituents, containing coded DNA or RNA which can, as one of their genetic functions, determine their own incorporation into specific vehicles for transmission to other cells"

DISTRIBUTION OF VIRUSES IN NATURE

The hosts of all the known viruses fall into four large taxonomic groups (1) flowering plants, (2) insects, (3) bacteria and (4) vertebrates All of the plants which are known to be affected by viruses belong to the classes of angiosperms, or flowering plants No viruses are known which attack lower plants or gymnosperms, but the possibility must be borne in mind that agents for the latter may have remained undetected because of lack of observation Among the angiosperms, the most thoroughly studied infections are in cultivated plants, such as sugar cane, peaches, tobacco, tomatoes and potatoes.

There are very few classes of bacteria for which bacteriophages have not yet been found (Luria, 1953), and they are groups in which technical difficulties in isolation and detection might be expected. Most of the phage found

When a phage-antibody mixture is diluted, one could expect some reactivation of neutralized virus if dissociation of the antibody-virus combination were at all a prominent reaction However, no reactivation takes place on dilution, and the phage-antibody union is regarded [redacted] nondissociable. This lack of reactivation on dilution is not due to any lethal effect of antibody on phage because the antibody can be digested away with papain (Kalmanson and Bronfenbrenner, 1943) or destroyed by sonic vibration (Anderson and Doermann, 1952) and the virus recovered in active form Antisera taken very shortly after the beginning of immunization of an animal are exceptional, and a combination between this early form of antibody and phage [redacted] moderately dissociable (Jerne and Avegno 1956) The explanation of this difference is not clear

Animal viruses, unlike phage, have no specific structure for attachment to the host cell, and there is no evidence so far that an injection mechanism is involved in infecting the cell It is not certain whether the entire virus particle or only part of it enters the host at the time of infection Investigation of the kinetics of neutralization with animal viruses has been hampered by the lack of suitable methods for measurement of surviving virus The older methods of titration in animals have the defect that the entire reaction mixture (virus + antibody) is brought into the host organism at the time of titration This means that the antibody introduced into the test animal has the possibility of continuing to react with virus during the incubation period of the neutralization test This defect is avoidable with the plaque method of virus titration

One of the first principles evolved from the study of virus serology was the "percentage law" (Andrewes and Elford, 1933) This law states that when virus is added to antibody and allowed to come to a state of equilibrium, the proportion (percentage) of virus surviving will be the same regardless of the amount of virus added This law holds reasonably well only when antibody is present in excess

With the plaque method of virus assay, it has been possible to eliminate some of the previous difficulties with studies on neutralization, and this has been used so far with poliomyelitis, Newcastle disease virus and Western

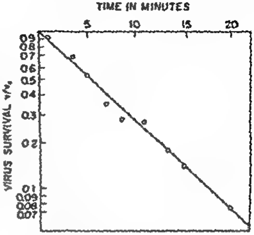

FIG 30 Curve of the kinetics of neutralization of WEE virus showing the residual active virus at different intervals after combining virus and antibody (Dulbecco, et al, 1956, A study of the basic aspects of neutralization of two animal viruses, Western equine encephalitis virus and poliomyelitis virus Virology 2, 162-205)

equine encephalomyelitis viruses With this method, one can study the kinetics of the virus-antibody reaction The rate at which antibody reacts with virus is shown in Fig 30 A small amount of antibody (Dulbecco et al, 1956) was added to the virus, and samples were withdrawn at intervals They were diluted immediately by a large factor so that virus-antibody union would be stopped at once, and the samples were tested for surviving virus The results show that during the inactivation of the first 95 per cent of the virus the proportional rate of inactivation remained constant

In extending this experiment, different concentrations of serum were added to the same amount of another virus, and again the disappearance of active virus with time was measured From this experiment (Figs 31 and 32) it can be seen that the rate of virus disappearance (slope of lines in Fig 31) [redacted] directly proportional to antibody concentration These two experiments show that, within the range investigated the logarithm of the per cent of original virus which remained active was inversely proportional to the time of incubation and to the concentration of antibody

of serology for studying macromolecules The property of infectivity makes it possible to detect single virus particles even at high dilutions, and this, in turn, makes it possible to study the kinetics of the virus-antibody reaction at concentrations which are unique and provide data of interest to virologist and immunologist alike Serology also provides a tool with which subtle differences can be detected between large molecules, differences which remain obscure even after chemical analysis Such a tool is invaluable in identifying the different components of virus particles, in distinguishing these components from host cell substances and in following quantitatively the production of virus subunits in an infected cell before such time as the parts are assembled into mature particles, recognizable by their ability to infect

There are four principal ways of measuring virus-antibody reactions (1) the precipitin or agglutination test; (2) neutralization, (3) complement fixation, and (4) hemagglutination inhibition. Each of these tests has its own areas of usefulness and, while it is conceivable that two of them could measure precisely the same antigen-antibody reactions, this seldom happens in actual practice

The Precipitin Test. Precipitin tests may be performed either with virus particles or on soluble substances found in virus preparations Virus particles may be made to agglutinate or precipitate by homologous antisera, but this requires a high concentration of particles There is a well-established relationship (Merrill, 1936) between the size of a particle and the concentration necessary to obtain visible aggregation or precipitation with antibody. The critical concentration for some animal viruses is as much as 10^{12} particles per ml, and this amount is usually prohibitive for extensive studies Recently, however, the Ouchterlony and Oudin methods for obtaining precipitates in agar gels have been used for virus serology, and this promises to be a useful technic. Plant viruses are frequently typed by the precipitin method, since they are often available in large quantities.

The Neutralization Test. Neutralization may be defined as the loss of the infectious property of a virus particle because of a reaction with antibody. Neutralization is of special interest to the virologist and to the clinician because it is the essence of a major protective mechanism in vertebrates against virus infections. Neutralization is of special interest to immunologists because the reaction offers an opportunity to study antigen-antibody reactions at concentrations which are unique The infective property of viral particles can be measured accurately even at very high dilutions, therefore, the effect of antibody can likewise be measured in concentrations where lattice formation and other events do not complicate the results.

Plant viruses can be neutralized with antiviral rabbit sera, but the kinetics of the reaction have been very little studied, partly because of technical complications, such as the neutralizing effect of normal sera, and partly because of the very low efficiency of normal particles in producing foci on leaves It is doubtful whether the mechanism of neutralization of plant viruses can be the same as that of animal and bacterial agents Animal and bacterial viruses are neutralized by absorption of antibody to attachment sites while, in plant viruses, there is no evidence so far of sites which facilitate the initial stages of infection

Bacterial and animal virus neutralization have so much in common that they can be discussed together profitably Coliphages stimulate formation of at least two antiphage antibodies (as shown by reciprocal absorption) (Lanni and Lanni, 1953), one for the head and one for the tail. An entire phage particle

ever, the absorption of even very large amounts of antibody on the body of the virus has no effect on the ability of phage to infect a cell by injecting its DNA into the bacterium Of the antibody absorbed to the tail, presumably only that absorbed at the tip has an effect in neutralization When phage is mixed with an excess of antibody and the course of inactivation is followed for several hours, it proceeds at an exponential rate, and this first-order reaction suggests that the inactivation of each particle is due to a single event—the absorption of an antibody molecule to a critical site The kinetics of animal viruses are similar and are discussed below.

infective agent by means of the absorption of a single antibody molecule This model will give first-order kinetics consistent with experiment (Figs. 30 and 31) There are two stages of neutralization: (1) the formation of noninfective but adsorbing particles, and (2) the formation of noninfective and nonadsorbing particles To convert a single site to stage 1 requires a single antibody molecule, and to stage 2 (with NDV) requires 2 to 3 molecules (Rubin and Franklin, 1957) To inactivate a virus particle requires that all sites react with antibody or else that the particle attach to the host at a site already occupied by antibody.

Dissociation of Antibody from Virus. Another problem of common importance to immunology and virology concerns the firmness with which specific antibody molecules unite with virus particles It is a matter of both practical and theoretical importance to know whether a virus particle, once neutralized, can be reactivated by spontaneous dissociation of antibody The view from classic immunologic work is that under physiologic conditions, spontaneous dissociation of antigen and antibody is negligible On the other hand, the ability of the virologist to detect activity in single particles at high dilution furnishes conditions for dissociation which have not been examined previously The studies on neutralization of phage by dilution indicate that the phage-antibody complex is completely nondissociable with most sera However, a great number of animal viruses appear to be reactivated by simple dilution of neutral mixtures, and this has led to the expression of the view that animal viruses as a class are *freely* dissociable (Burnet, 1955) Recent evidence makes this conclusion doubtful

A typical example of virus reactivation by dilution is as follows A large amount of influenza virus is to be tested in the allantoic sac Sufficient antiserum is added until the injection of virus and antibody fails to give detectable hemagglutination after incubation Such virus is said to be neutral and operationally, this means that an insufficient number of cells were infected with virus to yield a detectable HA titer However, if the so-called neutral mixture is diluted 10 or 100 times and the same volume (0 1 ml) used to inoculate the allantoic sac, detectable hemagglutinins are produced (McKee and Hale, 1946)

Two interpretations of this experiment are possible (1) The virus yield from the diluted inoculum was due to antigen-antibody dissociation Antigen-antibody dissociation is marked and, when the reagents are diluted, the reassociation reaction rate is reduced markedly, and an increase of active virus results (2) An alternative explanation is that such dissociation is minor or negligible. The so-called neutral mixture actually contains particles which infect, but the presence of large amounts of uncombined antibody in the allantoic sac (from the inoculum) prevents any significant amount of cell-to-cell spread of these infections However, when the "neutral" mixture is diluted, essentially less of the uncombined antibody is put in the allantoic sac The few cells that are infected can infect other cells more efficiently, and a detectable yield results

The deficiency of this technic (the transfer of antibody to the test organism) does not permit direct proof of the dissociation theory, and most animal virus neutralization experiments suffer from this same defect When dissociation-dilution experiments with animal viruses are done by the plaque assay method, this pitfall may be avoided With this technic, the degree of dissociation found is at best very small (Dulbecco et al, 1956), and the results are not radically different from what is obtained with phage If the results with poliomyelitis, WEE and NDV are typical, then dissociation with animal viruses is minor Mandel (personal communication) has shown with poliomyelitis that a very small portion of neutralized virus is dissociable by dilution under physiologic conditions, even in the absence of cells

Another method of dissociating antibody is by displacement of active with inactive virus in a virus-antibody complex Active virus is neutralized, and a great excess of inactivated virus (heat or UV inactivation) is added As a result, active virus appears in the previously neutral mixture This is also clear evidence of at least a low grade of virus-antibody dissociation (Dulbecco et al, 1956) These experiments also show that the virus is not fundamentally altered by the antibody.

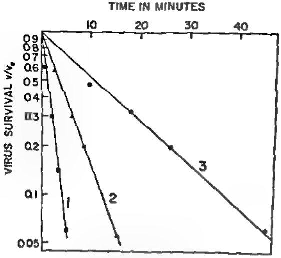

Fig 31 Change in the slope of the kinetic curve for neutralization of poliomyelitis virus (type I), using serum concentrations in a ratio of 10/3 3/1 for curves 1, 2 and 3. (Dulbecco, et al, 1956, A study of the basic aspects of neutralization of two animal viruses, Western equine encephalitis virus and poliomyelitis virus, Virology 2, 162-205)

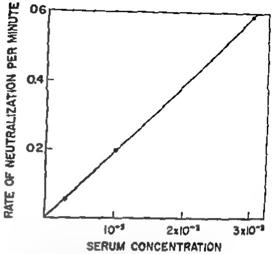

Fig 32. Data taken from Figure 31 in which the rate of virus neutralization per minute is plotted against serum concentration

This relationship may be expressed in the following equations (Luria, 1953).

$$\frac{P}{P_o} = e^{-ktC} \qquad \log_e \frac{P_o}{P} = ktC \qquad \log_{10} \frac{P_o}{P} = 0.43ktC$$

Where "P_o" is the initial virus concentration, "P" is the fraction remaining active, "t" = the time of contact between virus and antibody, and "C" = the concentration of antibody, "k" is a constant which is proportional to the fractional rate of inactivation and is characteristic for a given virus and a given serum. This also expresses the consequences of the percentage law for the case where "C" is large and "t" is long

When the same serum is used with different viruses, the "k" values are an expression of the degree of relatedness of the two agents. Antiserum prepared against one virus naturally gives a low slope with a relatively unrelated agent.

One important feature of the curves in Figures 30 and 31 is that they are straight lines through the origin This is typical of a first-order reaction and strongly suggests that the neutralization of a virus particle is due to a single event—the combination of a molecule of antibody with a critical site on the virus If more than one antibody molecule were *required* for inactivation, it would be expected that the initial rate would have been slower than the final rate The occurrence of a single critical site on a virus particle seems a bit improbable on purely intuitive grounds, and actually this type of curve does not exclude multiple attachment sites on a particle, as will be explained below.

From data on NDV neutralization (Rubin, 1957) and other sources, a likely model of neutralization is as follows: A virus particle has multiple attachment sites, and the number in the case of an NDV particle has been roughly estimated at 4 to 6 (Rubin, 1957) If a single antibody molecule adsorbs to an attachment site, the absorbing area is not entirely covered, at least functionally, and the virus may still attach to the host cell by means of this site, but since penetration and infection do not take place, such a particle is scored as neutral However, if several antibody molecules react with an attachment site, the area may be so covered that the virus can no longer adsorb to a host cell at this location Thus, each particle *may* be eliminated as an

detection and the identification of such components The term "soluble substance" is used to indicate viral products or by-products of virus growth which are smaller in size than the virus itself. They are often released by the virus-infected cell The soluble substances are quite different from one virus to another. In the case of vaccinia virus, the soluble substance is a protein which can be separated from the elementary virus particle without loss of virus viability while, in the case of influenza virus, it is a nucleoprotein which probably is completely covered in the intact particle but is produced in free form by infected cells Such substances can be detected and quantitated by serologic methods such as complement fixation

The antigens which make up the mature particle are manufactured by the infected cell as subunits which are later assembled into mature particles. By an elegant extension of conventional serologic methods, fluorescent antibody can be used to locate virus constituents in the infected cell before the final assembly has begun This is discussed in detail in the section on animal virus multiplication

Serologic tests may be used for fairly fine but usually not very precise comparisons of closely related but different virus strains It is widely recognized, for example, that the serologic properties of influenza A viruses differ from one outbreak to another By the use of serologic tests, a number of closely similar strains may be tested for their ability to react with a corresponding set of antisera Reciprocal tests can be set up between the strains and their antisera and, even if the differences are slight, each strain may show a higher affinity for its own antiserum The differences can be magnified by the use of cross-absorbed sera The small distinctions which can be made are useful, especially with influenza where naturally occurring minor modifications in the virus are common (Jensen, 1957) and important

HEMAGGLUTINATION

During recent years, it has been found that a wide variety of animal viruses are capable of *agglutinating red blood cells, and hemagglutination*, like serology, has become a useful tool in both the clinical and the experimental laboratory The first example of virus hemagglutination (Hirst, 1941; McClelland and Hare, 1941) was found accidentally in the course of work with allantoic fluid from chick embryos that had been infected with influenza virus Whenever allantoic fluid is casually removed from an egg, it becomes mixed with freshly shed embryonic blood, and this afforded an excellent opportunity to see the agglutination which takes place almost at once in those fluids that contain a great deal of virus It was found subsequently that this clumping was due to the direct action of the virus particle on the red cell, and similar examples involving other viruses soon followed The importance of hemagglutination is *broadly 2-fold*, *(1)* a technic for in vitro titration of viruses and antibodies, and (2) a model for host-virus interactions

The viruses which are known to cause hemagglutination fall into several well-defined groups, plus a number of miscellaneous and isolated examples:

1 [illegible]
are 2 [illegible]
plex [illegible]
vaccir [illegible]
[illegible]

only group in which the hemagglutinin is clearly and readily separable from the intact infectious virus particle

2 The arbor virus group This is made up of arthropod-borne viruses, such as the agents of Japanese B encephalitis (Sabin and Buescher, 1950), yellow fever, West Nile, dengue, St Louis and others (Casals and Brown, 1954) This group has been subdivided into 3 parts (A, B and C) on the basis of antigenic relationships as determined by the hemagglutination inhibition test (Chap. 12)

[illegible]

thetic group from certain mucoproteins, which is a reaction having a bearing on adsorption and possibly penetration

In summary then, neither animal nor bacterial viruses show much of a dissociation effect when tested by similar methods, and it is not necessary to invoke dissociation for most reactivation experiments on the dilution of animal virus-antibody mixtures.

The Persistent or Non-neutralizable Fraction. The phenomenon of the persistent virus fraction can be illustrated in the following experiment Different amounts of antibody (Fig 33) are added to a constant amount of poliomyelitis virus and, after a period of incubation, the surviving virus is measured by plague assay. With small amounts of antibody, the logarithm of the surviving virus fraction varied inversely with the serum concentration However, when the surviving fraction became less than 1 per cent, the addition of a large excess of antibody had no effect in decreasing it further This residue of active virus is known as the persistent or non-neutralizable fraction It also occurs with other

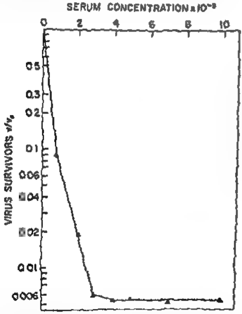

FIG. 33 Curve of neutralization of poliomyelitis virus, using different concentrations of serum and 4×10^7 plaque-forming units of virus Virus and serum stood for 2 hours before virus survivors were plated. (Dulbecco, et al, 1956, A study of the basic aspects of neutralization of two animal viruses, Western equine encephalitis virus and poliomyelitis virus, Virology 2, 162-205)

virus-antibody systems but is especially prominent with poliomyelitis virus

The following statements may be made about this fraction of virus: (1) It is not genetically different from the bulk of the virus in a preparation. Subculture of resistant virus does not give resistant virus (Dulbecco et al., 1956). (2) The virus particles are neutralizable. They do not have a different type of coat than the majority (Mandel, unpublished observations). (3). The persistent fraction seems to come from virus which has reacted with antibody and, after adsorption to the cell, penetrates slowly During this period of slow penetration the process of infection may be stopped if the virus reacts with more antibody or, if the already attached antibody dissociates, the virus may go ahead and infect (4) The persistent fraction is probably due to an equilibrium state between antibody, virus and antibody-virus complexes partly free and partly adsorbed to the cells

There are at least two stages in the adsorption of an animal virus to a host cell The first is characterized by loose binding, and the virus remains accessible to the neutralizing action of immune serum This goes rapidly into a second stage in which the adsorption is no longer reversible nor is it affected by serum The rate at which the virus goes from the first to the second stage is affected markedly by temperature The fact that antisera have no effect on viruses after an early stage of adsorption or penetration has important implications for the pathogenesis and the treatment of virus diseases Antisera may affect the ability of virus to move from one cell to another but cannot affect the issue in a cell already infected

In addition to antibodies directed at the virus particle it is also possible to work with antibodies directed at the host cell, especially in in vitro systems (Quersin-Thiry, 1955, Habel et al, 1958) This aspect of serology has as yet been explored very little but appears to be important Some anticell antibodies are capable of preventing infection with certain viruses but not with others.

Serologic Detection of Virus Antigens. Viruses are commonly made up of a number of macromolecular components which can often be fractionated by relatively simple procedures Serology is a useful tool for the

detection and the identification of such components The term "soluble substance" is used to indicate viral products or by-products of virus growth which are smaller in size than the virus itself. They are often released by the virus-infected cell. The soluble substances are quite different from one virus to another In the case of vaccinia virus, the soluble substance is a protein which can be separated from the elementary virus particle without loss of virus viability while, in the case of influenza virus, it is a nucleoprotein which probably is completely covered in the intact particle but is produced in free form by infected cells. Such substances can be detected and quantitated by serologic methods such as complement fixation.

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1 [illegible]
are 2 [illegible]
plex [illegible]
vaccin [illegible]
1944) ectromelia (Burnet and Boake, 1946) and (B) the psittacosis-lymphogranuloma venereum group of viruses including meningoencephalitis (Hilleman et al., 1951) and psittacosis (Gogolak and Ross, 1955) This is the only group in which the hemagglutinin is clearly and readily separable from the intact infectious virus particle

2. The arbor virus group This is made up of arthropod-borne viruses, such as the agents of Japanese B encephalitis (Sabin and Buescher, 1950), yellow fever, West Nile, dengue, St Louis and others (Casals and Brown, 1954) This group has been subdivided into 3 parts (A, B and C) on the basis of antigenic relationships as determined by the hemagglutination inhibition test (Chap 12)

3 The myxovirus group. This includes the viruses of influenza (subtypes A to D), mumps (Levens and Enders, 1945), Newcastle disease (Burnet, 1942) and fowl plague (Lush, 1943) This is the group of greatest general interest in virology because all members have an enzyme capable of splitting off a small prosthetic group from certain mucoproteins, which is a reaction having a bearing on adsorption and possibly penetration

4. Echo viruses and Coxsackie B_3 The occurrence of a fourth major group of viruses which cause hemagglutination has been described recently (Goldfield et al., 1957, Labelle, 1958) and includes a number of echo viruses (types 3, 6, 7, 10, 11 and 12) as well as Coxsackie B_3 The hemagglutinin is active against human O cells, and the reaction can be inhibited by strain-specific antisera Of greatest interest is the information, so far available only in preliminary form, that the hemagglutinin is part of the virus particle, and that the HA activity elutes from red cells after destruction of red cell receptors. A receptor gradient similar to that seen with myxoviruses was found, and the receptors for echo and myxoviruses are entirely different

5 Adenoviruses Agglutination of erythrocytes by adenoviruses has been described recently by Rosen (1958). More than 18 different types were tested against a variety of species of red cells and all but 3 agglutinated at least a species of cell The pattern in terms of the cell species agglutinated appeared to be specific for each virus type.

6. The miscellaneous group.

A. The encephalomyocarditis viruses (Hallauer, 1946; Ohtsky and Yager, 1949), including strains Columbia-SK, MM, EMC and Mengo These agents agglutinate only sheep cells

B Pneumonia virus of mice (Mills and Dochez, 1944, Curnen and Horsfall, 1947)

stable and dissociates from tissue inhibitors at high temperatures

C The GDVII strain of murine poliomyelitis (Labelle and Horsfall, 1949) This is the only known strain of this group which has a hemagglutinin It agglutinates human cells only at low temperatures

D Polyoma virus of mice (Eddy et al, 1958). This is an example of a tumor-inciting virus which agglutinates red cells of several mammalian species

Hemagglutination by Lipoproteins

A Pox viruses Vaccinia, variola and ectromelia all produce dermal vesicular infections, they are antigenically related, they are of similar size and constitution, being large and complex as viruses go, and they all have a hemagglutinin which is not an integral part of the virus The hemagglutinin can be separated readily from the virus, either by centrifugation or by absorption with susceptible red cells, and this can be done without significant loss of virus infectivity. Vaccinia

and Boake, 1946). Both of these hemagglutinins act alike in agglutinating the red cells of about one half of the ordinary domestic fowl Neither agglutinates chick embryo cells, and only ectromelia is active on mouse red cells.

The hemagglutinins of this group can be inactivated by the action of both the *B. welchii* alpha toxin and by cobra venom if these reagents are used under conditions where the lecithinase they contain is active (Stone, 1946). Purified mixtures of lipids also agglutinate the same fowl cells that are agglutinated by viral preparations. These bits of evidence point to the likelihood that the hemagglutinins are phospholipids The serologic specificity of ectromelia and vaccinia hemagglutinins makes it seem more likely that the molecule is complex, possibly a lipoprotein

There are quantitative differences in the amount of hemagglutinin produced in different hosts and, in infected calf skin, it appears to be absent, although this may be due to destruction by oxygen from the air (Stone and Burnet, 1946). Some strains of vaccinia do not produce a hemagglutinin, nor do animals infected with such strains produce antibodies against the hemagglutinin of other strains (Fenner, personal communication) Animals infected with hemagglutinating strains do produce antibodies against the hemagglutinin, and it has been shown that these are not neutralizing antibodies The function of the hemagglutinin in infection is not clear

B The psittacosis-lymphogranuloma venereum group. The viruses of this group are large and complex and, like the pox viruses, they tend to produce chronic low-grade infections and are susceptible to the action of antibiotics Of the viruses in this group, murine and feline pneumonitis, meningopneumonitis and psittacosis are known to possess a hemagglutinin. Only psittacosis virus (a single strain) has been studied in any detail (Gogolak and Ross, 1955). The results are strikingly similar to what was found with the pox viruses The active fraction is readily separable from the virus particles by simple centrifugation The hemagglutinin is active only against mouse cells The active material contains a lipoprotein from which a lipid may be extracted which agglutinates red cells but has

no serospecificity, while a nucleoprotein extracted from the same material has antigenic specificity but no action on red cells Lecithinases will destroy the hemagglutinating activity. Antiviral antibody specifically inhibits the hemagglutination reaction, but psittacosis and meningopneumonitis could not be differentiated by this method

Hemagglutination by Arthropod-Borne Viruses This group includes a number of small viruses which can multiply in vertebrate hosts and are also found in and may be transmitted by mosquitoes The hemagglutinins are usually prepared from the brains of young mice, and the extracts are most active on the red cells of newly hatched chicks The agglutination reaction must be carried out under rather sharply prescribed conditions It is inhibited readily by lipids and is very sensitive to slight changes in pH Few studies have been done on the hemagglutinin itself or its relationship to the virus particle The viruses which have this activity include many of the encephalitic agents: St Louis, Murray Valley, Japanese B, Western equine, Russian spring-summer and others The active group also includes the viruses of dengue and yellow fever, as well as a number of other agents that have been isolated directly from mosquitoes These agents differ from each other antigenically, and in neutralization tests the differences are frequently found to be large Antigenic disparity is somewhat less by the agglutination inhibition test and, on the basis of the latter, all of these viruses have been subdivided into 3 large fairly homogeneous groups (arbor A, B and C) (Casals, 1957)

Hemagglutination by Encephalomyocarditis Viruses The viruses of this group incl[illegible]

vir[illegible]

she[illegible]

from the red cell at high temperatures, and the red cell receptors are unaffected by contact with the virus. The attachment of virus to cells will not take place in the absence of calcium It has been reported that crude extracts (Verlinde et al, 1951) containing the receptor-destroying enzyme for the myxoviruses also act on receptors for this group, but there is scant evidence that the same

[illegible]

poliomyelitis virus (GDVII) is known to agglutinate red cells, though other closely allied and antigenically related strains have been tested. The GDVII strain agglutinates human red cells at low temperature but not at 37° C., where the virus dissociates from the receptor. The red cells thereafter are normal in appearance and are reagglutinable. The hemagglutinin is clearly part of the virus particle and is inhibited by antiserum A mucopolysaccharide found in mouse intestine (Mandel and Racker, 1953) has both a hemagglutinin inhibiting and a neutralizing effect on this strain The polysaccharide may be dissociated from the virus by changing the salt concentration, but the inhibitory action of the carbohydrate is not destroyed by the virus This mucopolysaccharide has no inhibitory effect on other viruses, such as those of the myxo group for example, but an intestinal virus combining with a polysaccharide overlying the host cells seems to be closely analogous to the influenza model A striking difference is the inability of GDVII to alter this polysaccharide in a detectable way

Hemagglutination by Pneumonia Virus of Mice (PVM) All virus strains known give the hemagglutinin reaction The virus agglutinates mouse and hamster cells The tissues of both these animals contain high levels of hemagglutinin inhibitor which combines rapidly with the virus, especially when an attempt is made to remove the agent from the lungs by grinding (Curnen and Horsfall, 1947, Curnen et al, 1947) The virus may be dissociated either from red cells or from tissue inhibitor by heating to 75° C. This temperature does not inactivate the hemagglutinin itself The hemagglutinin is part of the virus particle It is possible, though unproved, that the hemagglutinin represents an attachment mechanism operative in normal infection

Hemagglutination by Myxoviruses There are 4 different viruses in this group (influenza, mumps, NDV and fowl plague [FPV]), and all the strains tested have the capacity of splitting neuraminic acid derivatives from mucoproteins which are found in body fluids or on the surfaces of cells These agents differ very markedly in the kind of disease picture they produce in their normal hosts For a variety of reasons, they have become model agents for study of animal virus behavior, and they are described further under the section on virus multiplication.

The viruses of this group agglutinate a variety of red cells, both avian and mammalian Not all mammalian cells agglutinate equally well but, in practice, human, guinea pig or domestic fowl red cells are usually

used The agglutination of red cells occurs rapidly under physiologic conditions and, when the ratio "particles/red cells" is high, visible aggregation occurs within a few seconds. The occurrence of clumping is due to the formation of a lattice structure in which virus particles form bridges between red cells

Agglutination or adsorption of virus to cells requires the presence of electrolytes, and it occurs almost equally well over a wide range of hydrogen ion concentrations and at either zero or 37° C. A number of lines of evidence indicate that the hemagglutinin is part of the virus particle itself The number of attachment sites per virus particle is not known except that it must be 2 or more. From data on their enzymatic activity, it may be supposed that the virus sites are protein in character Red cell receptors are also multiple, they are mucoprotein in nature, and more than 1,000 virus particles may attach to a single cell

The clumping of red cells has been widely used in a test for the presence of virus Since the property of hemagglutination is more stable than that of infectivity, the hemagglutination titer gives a closer estimate of the number of particles than of the number of infectious units Of course, the sensitivity of the method does not compare with that of infectivity With a well-adapted laboratory strain, prepared in the best way, it requires of the order of 10^8 infective particles to cause ... con- ... ual 10 to 20 inactive particles for every infective particle, or about 10^7 particles per ml for visible agglutination Estimates of the number of hemagglutinating particles can also be made by counting the number of red cell dimers formed when red cells are present in excess of the virus Each dimer corresponds to 1 virus particle (Levine et al, 1953, Horsfall, 1954). Still another method for estimating particle number makes use of the adsorption to red cell ghosts A virus preparation is allowed to adsorb to ghosts and the number of adsorbed particles per ghost can be counted by electron microscopy (Dawson and Elford, 1949)

Hemagglutination can be inhibited by specific antibody directed at the virus. There is a considerable body of evidence that antibody which is antihemagglutinating is also neutralizing With influenza, there is a fairly close though not perfect correlation of inhibiting titer with neutralizing titer in a wide range of sera. The kinetics of neutralization follow very closely the kinetics of hemagglutination inhibition with NDV (Rubin, 1957). With influenza, both inhibition and neutralization show a high degree of strain specificity, in marked contrast with what is shown by the complement-fixation reaction However, absorption experiments indicate that the overlapping is not complete, and there is some antibody which neutralizes but does not inhibit (Walker and Horsfall, 1950) By and large, it appears that the sites by which a *virus attaches to a red cell are the same as* those through which it attaches to a host cell, and antibody which interferes with one operation will interfere with the other.

Operationally, the viruses of this group are distinguished from all others by the fact that they elute *spontaneously* after adsorption to red cells The cells that release virus after adsorption simultaneously lose virus receptors and, eventually, come to a state where they do not adsorb virus at all. On the other hand, the virus particles are capable of undergoing numerous adsorption-release cycles without detectable change There is now considerable evidence that the viruses of this group have an enzyme capable of destroying an essential part of the cell receptor, and this destruction is what permits elution to occur The nature of the enzymatic reaction is now known and is discussed in a later section

The Action of Viruses on the Red Cell. When influenza viruses are allowed to act on red cells until all the receptors are removed, there are no visible changes Virus action uncovers a new antigen on the red cell surface (Burnet and Anderson, 1947) The electrical charge of the cell changes markedly as receptor destruction continues (Hanig, 1948).

Studies on these changes in red cells were greatly facilitated by the discovery of an enzyme in bacterial filtrates (Burnet and Stone, 1947) which is like the virus enzyme of this group in that it destroys cellular virus receptors The enzyme is widespread in the bacterial world and is commonly prepared from *V. cholerae.* The discovery of this receptor-destroying enzyme (*RDE*) proved to be especially important in studying the receptor gradient and the influence of receptor destruction on infection of cells

The Receptor Gradient. When red cells are treated with the bacterial enzyme RDE,

TABLE 6

CELLS TREATED WITH VIRUS STRAIN	AGGLUTINATION WITH VIRUS AFTER TREATMENT						CHANGE IN MOBILITY AFTER TREATMENT
	Mumps	NDV	Mel	PR8	Lee	SW	
Control (untreated)	++	++	++	++	++	++	1.30
Mumps	−	+	++	++	++	++	1.18
NDV	−	−	−	++	++	++	0.50
Mel (Inf. A)	−	−	−	++	++	++	0.80
PR8 (Inf. A)	−	−	−	−	++	++	0.74
Lee (Infl. B)	−	−	−	−	++	++	0.74
Swine	−	−	−	−	−	−	0.37
RDE (Enzyme)	−	−	−	−	−	−	0.17

The cells were exhaustively treated with the viruses shown on the left and then tested for agglutinability by the viruses shown at the top

− = no agglutination

+ = partial agglutination

++ = full agglutination

The electrophoretic mobilities of the cells after treatment (from Ada and Stone, 1950) are shown on the right.

virus receptors are gradually removed and, after a time, the cells will no longer be agglutinable by any of the myxoviruses. When the agglutinability of cells is tested with a number of myxoviruses during the course of RDE treatment, it is found that they lose their ability to be clumped by (or to adsorb some of the viruses long before their reaction with other viruses is much affected. The order in which the viruses lose agglutinating power is called the receptor gradient (Burnet, 1942, 1955) (Table 6). The same gradient may be demonstrated by using some of the viruses to remove receptors. Strain *Swine*, for example, which is far down the gradient and agglutinates RDE-treated cells after most other strains fail, is also a potent virus in removing all of the receptors. Other strains in the gradient series are less vigorous and, in general, will abolish receptors only for themselves and for those viruses which are higher in the gradient. When very large amounts of virus are used in treating red cells and the incubation is carried out over a long period of time, all receptors may be removed, even by a virus high in the series (Hirst, 1950).

The removal of receptors from red cells, either by viruses or by RDE, is accompanied by a marked change in the electrophoretic mobility of the cells (Hanig, 1948). In general, it is found that the change in mobility and receptor removal run parallel. Using RDE to remove receptors, it is possible to determine the mobility of cells at the stages when agglutinability for various viruses disappears (Ada and Stone, 1950, Stone and Ada, 1950, 1952). The same parameters can be followed when various viruses are used for removing receptors. In general, it has been found that those strains that remove few receptors can change the mobility relatively little, and vice versa (Table 6). However, the mobility at which agglutinability with a given virus ceases is variable, depending on what agent is used to remove receptors. Other anomalies are also found. For example, Mel virus removes mumps receptors early, before the cell mobility is greatly lowered, while RDE does not remove them until the cell mobility is very low.

A satisfactory explanation of the receptor gradient obviously involves complexities of virus and cell structure with which we are not yet able to cope. Two general approaches to an explanation have been offered: (1) The first hypothesis involves the assumption that there are at least several different kinds of receptor spots and several kinds of virus enzymes. The gradient would be due to the presence of different arrays of these enzymes

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PR8 (Infl A)	−	−	−	−	++	++	0 74
Lee (Infl B)	−	−	−	−	++	++	0 74
Swine	−	−	−	−	−	−	0 37
RDE (Enzyme)	−	−	−	−	−	−	0 17

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on different viruses Just from the data in Table 6, it appears that the number of qualitatively different receptors might have to be rather large, and the main difficulty with this approach is that independent evidence for this multiplicity of enzymes and receptors is lacking. (2) The second hypothesis assumes that the red cell receptors are qualitatively similar but vary in their accessibility to enzyme action In this case, the viruses would differ in their ability to lyse the less accessible groups This theory has an *ad hoc* quality which is not very satisfying, besides which it does nothing to explain all the anomalies of agglutination and mobility

Removal of NDV Receptors. It seems reasonably clear that the forces which bind virus and red cell together are in some way related to an enzyme-substrate combination, and when the substrate is altered the binding force disappears, and virus enzyme and substrate separate It was first shown clearly by Sagik et al (1954) that one virus particle could remove all of the receptors from a cell without separation at any time The results suggest that the particle is adsorbed to the cell at several sites simultaneously and, as one receptor is lysed, the virus rolls around until it makes contact at another point This process is continued until all available receptors are used up This method of receptor destruction (sometimes called browsing) is doubtless more efficient than a process wherein virus becomes fully detached after each enzyme-substrate reaction It seems possible, though unproved, that the cessation of receptor lysis found by Stone and Ada may have coincided with the end of lysis by browsing and that the receptors remaining thereafter were removed at a much slower rate (Hirst, 1950), one receptor per attachment.

Hemolysis. One other change which sometimes goes with the action of viruses of this group on red cells is hemolysis. Both mumps and NDV cause partial hemolysis of cells, but it may well be that this is due to a system apart from that causing receptor destruction. All the evidence indicates that it is necessary for the virus to be attached to the cell by the regular receptor sites in order for hemolysis to occur. Preliminary destruction of red cell receptors, either by RDE or by influenza viruses, prevents lysis by mumps and NDV (Burnet and Lind, 1950; Chu and Morgan, 1950).

Agglutination by Freshly Isolated Influenza Virus. When some strains of influenza virus are first isolated from the human respiratory tract (Burnet and Bull, 1943) by inoculation of the amniotic sac, it is found that the infected amniotic fluid agglutinates guinea pig (or human) red cells to high titer but does not agglutinate chicken red cells, or at least the titer is low. After a single further passage in the allantoic sac of chick embryos, the virus is capable of agglutinating *both* kinds of cells to high titer, and this is the usual form (D or derivative) of most laboratory strains If the virus is passed in human embryonic cells (Mogabgab et al, 1954), it may remain in the O form This can also be done in the amniotic sac with some care Studies suggest that the D form is a mutant which is selectively favored with growth in the allantoic sac and suppressed somewhat in human host cells, but the physiologic significance of the altered agglutination pattern is not known

Hemagglutination Inhibitors. Ever since the enzymatic nature of receptor destruction was suggested, the elucidation of the chemical nature of the reaction has been a challenge No real progress toward this end was made as long as the substrate being studied remained part of a large cell A crucial step in further progress was the discovery that influenza virus could be heated in such a way that it retained its ability to attach to receptors and agglutinate red cells but lost its power to destroy receptors and elute from cells (Francis, 1947) The virus behaved very much as if it had retained much of the receptor configuration but had lost some part of the enzymatic mechanism With this type of virus, it was discovered that substances were present in many body fluids and secretions (Anderson, 1948) which also (like red cell receptors) combined with heated virus irreversibly so that the virus could no longer agglutinate red cells It was suspected and confirmed that these substances were analogous to the surface material of red cells responsible for virus attachment. Unheated virus, for example, could destroy the effect of these soluble inhibitors. A soluble inhibitor

has been extracted from red cells (Howe et al., 1957).

These substances which gave inhibition came from a variety of sources secretory glands such as the salivary or parotid, ovarian cysts, egg white, serum, urine, tissue extracts and others In every case where it has been sufficiently investigated, the inhibitory substance has proved to be a mucoprotein Mucoproteins are a class of compounds in which a protein moiety is coupled with a large number of polysaccharide units. They are notoriously difficult to purify, and one of the best sources of pure inhibitor has been from urine where there are relatively few other proteins (Tamm and Horsfall, 1950)

In every case that has been examined, the action of either RDE or one of the myxoviruses on inhibitors has resulted in the loss of inhibitory capacity and also in the release of small molecular weight compounds which include N-acetyl neuraminic acid (NANA) other similar reducing sugars and amino acids (Gottschalk, 1957). From cow colostrum a trisaccharide (neuramin lactose) has been isolated and from partial degradation of natural mucoidal HA inhibitors N-acetyl chondrosamine has been obtained as a disaccharide Neither of these compounds is an inhibitor but the virus releases NANA from both of them In the former, the NANA is believed to be attached to the hydroxyl group of galactose and, in the latter possibly to the nitrogen of the chondrosamine Gottschalk has suggested that the enzyme hydrolytically cleaves a glycosidic linkage joining the keto group of neuraminic acid to the H group of galactose or the nitrogen of chondrosamine This very exciting demonstration of the chemical action of a virus enzyme doubtless will lead in time to a deeper understanding of the agglutination process and the role of the enzyme in infection. The liberation of amino acids and other reducing substances from mucoids by virus action, as well as the complexities of the receptor gradient, strongly suggest that other virus enzymes and substrates may be involved in the destruction of receptors and inhibitors

In spite of the extent that viruses have been studied, very few intrinsic enzymes have been found. It is now widely assumed that, in their extracellular form, viruses do not contain any metabolic enzymes An ATPase has been found in preparations of avian erythroblastosis virus (Beard et al, 1955) and is believed to be part of the virus particle Enzymes which have been extracted from the tail of phage particles are able to attack the bacterial membrane (Kozloff et al, 1957; Weidel and Primosigh, 1957) Some bacteriophages which attack Friedlander's bacillus carry a specific hydrolytic enzyme which attacks the bacterial capsular substance (Adams and Park, 1956) and may assist the virus in clearing a path for attachment to the cell wall Phage-induced enzymes capable of cell lysis have also been found (Murphy, 1957)

FUNCTIONAL SIGNIFICANCE OF THE RECEPTOR-DESTROYING SYSTEM

It is somewhat paradoxical that more is now known about the chemical nature of the myxovirus enzyme-receptor system than about its functional significance for virus infection. It seems almost certain that it has a function, since all viruses of this group possess the enzyme, and all known host cells have lysable receptors

If it were not for the occurrence of receptors and the attachment system of viruses that goes with it, it is very likely that infection would not take place or that the efficiency of infection would be greatly reduced When host cells (mouse lung chorio-allantoic membrane or tissue culture preparations) are treated with RDE, infective virus no longer attaches very successfully and the probability of infection by virus is reduced sharply (Stone, 1948) In the living egg, receptors can regenerate with time, and the tissues resume their normal susceptibility All these facts indicate that intact receptors are either necessary or at least very helpful in bringing about infection While it seems logical that the next step after attachment would involve destruction of the receptor and penetration there is no direct evidence supporting this assumption Experiments have been described which purport to show that virus can enter the cell in the face of receptors which are resistant to lysis, but these experiments are not very convincing It has also been suggested (on the hypothesis that a drug, AMPS,

is a specific inhibitor of the virus enzyme) that the virus needs the enzyme for final separation from the cell after maturation as well as for initial penetration (Ackermann and Francis, 1954) It is also possible that the enzyme ■ necessary for some intracellular function Still another possibility is that the respiratory mucosa is covered by substances, *including mucoproteins, which attract and* bind influenza virus (such as the intestinal polysaccharide which binds mouse poliovirus [Mandel and Racker, 1953]) and would prevent it from infecting respiratory cells if it were not for the enzymatic ability of the virus which enables it to destroy this "defense" mechanism

In summary, hemagglutination has a wide distribution in the animal virus world With most viruses, however, the reaction between virus and red cells is an adsorption, dependent on electrolytes, often dissociated at high temperatures, specifically inhibited by viral antisera, but which has told us very little so far about virus-cell interactions which occur on infection Only *with the myxoviruses, where* the red cell-virus interaction is clearly analogous to host cell-virus interaction, has the study of the reaction given evidence which applies to the initiation of the infective cycle

VIRUS MULTIPLICATION

Viral multiplication is strictly intracellular Hence, we must consider as its essential process the production of viral materials within virus-infected or virus-carrying cells this process ■ generally only observable insofar as it culminates in the production of infectious virus *It is most easily studied when it does* so within a relatively brief span, within the very cell that has been infected . Even then, however, viral multiplication is a process radically different from the multiplication of cellular organisms, whether free living ones or intracellular parasites. The dependence on the host is . not purely a nutritional one, as for other parasites, but an integrative one We may speak of viral multiplication as *viral biosynthesis, in the* same sense as we speak of the biosynthesis of macromolecular cellular constituents and organelles This viewpoint need not rob viruses of their "independence," but interprets it as a genetic and evolutionary independence, limited by the demands and the implications of the integration process within the host cell (quoted from Luria, 1958).

In the sections which follow, the process of virus infection and multiplication is described for representative strains of bacterial, animal and plant viruses. These events will be considered in terms of the following steps: adsorption, penetration, change to the noninfective form, replication of virus subunits, assembly of subunits or maturation, extrusion and cell lysis The description is in terms of model viruses in each case, noting exceptions with other material where the facts are clear

Multiplication of bacterial viruses is discussed briefly, and for comparative purposes. The discussion and references are covered much more adequately in Chapter 7

Multiplication of Bacterial Viruses

The model system has been 7 coliphages (T series), all of which lyse a single strain of *E coli*, strain B (Luria, 1953). The phages are polygonal in shape and have tails of varying length, dependent on the strain. The polygonal head consists mainly of DNA and is surrounded by a protein membrane. The tail is also of protein and is different from that which envelops the head

Phages attach to the host bacterium by the *tip of the tail The bacterial site of attachment* is very specific and is a substance under the genetic control of the organism. The configuration of this site is subject to change by mutation, following which the phage will no longer attach and can no longer infect It ■ technically easy to obtain a variety of resistant bacterial mutants

The initial attachment of virus to host occurs after random contact and is a loose and reversible adsorption (Tolmach, 1957) This stage is followed rapidly by a reorientation of the virus to a more specific and irreversible attachment For both these stages there are minimal and specific electrolyte requirements For some phages other cofactors are also required for firm attachment (Stent and Wollman, 1950), of which the most efficient is 5-methyl-tryptophane. Following oriented attachment there is some hydrolysis of the bacterial membrane (Kozloff et al, 1957), and the injection mechanism is triggered so that the DNA content of the phage head is in-

jected into the bacterium An empty protein bag is left outside the cell and may be removed mechanically without altering the course of the intracellular infection (Hershey and Chase, 1952). This special device for infecting a cell is seen only with bacterial viruses and probably was necessitated by the rigid membrane which surrounds these cells It is interesting that, when the structure of the virus is injured, the particles are unable to infect bacterial cells unless the membrane is removed (Fraser et al., 1957)

After the infecting phage has injected its DNA, the cells may be broken open, and no infectious particles are found, confirming the observation that the entire virus particle does not enter the cell (Doermann, 1952, 1953) This is called the eclipse phase and lasts about 10 minutes with some strains If the cells are broken open at intervals after the eclipse phase, an increasing number of mature (infectious) particles are found The number increases linearly with time until the host cell bursts A burst may contain 30 to several hundred particles, depending on the virus strain During the latent period, a series of processes are going on, resulting in the replication of phage subunits which are assembled later into finished particles

In many cases, when phages are first absorbed the cell is killed, at least in the sense that it does not divide With the injection of virus DNA, the bacterial nuclei begin to disintegrate with margination of chromatin (Luria and Human, 1950) However, the over-all metabolism of the cell is unchanged (Cohen, 1947, 1949) in terms of respiration, glucose and phosphate uptake The energy-mobilizing apparatus appears to be intact but there are extensive changes in the specific tasks which are being carried out Synthesis of *bacterial* DNA, RNA and protein cease abruptly, but synthesis of viral protein and of viral DNA begin Head and tail proteins appear in the cell before mature particles are seen by electron microscopy and at a time when DNA strands (Kellenberger and Séchaud, 1957) can be seen in the cell Finally, mature particles appear

The biochemical evidence indicates that the synthetic capabilities of a cell remain intact at the level of energy mobilization, but the control of what is synthesized is shifted abruptly from guidance by bacterial DNA to virus DNA While this new activity is directed especially toward the manufacture of macromolecules, the shift also occurs at levels of synthesis of smaller elements Examples are the induction of thymine synthesis by phage infection of a thymineless mutant of *E. coli* or the induction of hydroxymethylcytosine synthesis by T_2 infection (Cohen, 1953, 1957). Except for the small contribution of the DNA of infecting virus, there are no large molecular weight precursors of phage DNA or protein which are incorporated as such from bacterial sources It is this sort of evidence which has disposed of the once widely held notion that virus production was the result of slight changes in pre-existent cell subunits

Relatively little is known of the mechanics of the assembly of phage from its basic subunits. Proflavine will prevent this maturation causing a yield of empty head membranes (DeMars et al., 1953, Levinthal and Fisher, 1952) These protein membranes are antigenically quite distinct from the tail proteins It is the protein antigens of the tail, at least those of the tail tip, which carry the host range specificity of the phage These tail antigens are controlled by a number of genetic factors When infection of a bacterium takes place with two related phages which have different tail antigens (labelled "A" and "B" here for illustration), then progeny particles can be found which have "A" tails but are genetically "B" i e, the progeny of the next generation would have "B" tails Other particles have "B" tails but are "A" genotypes, and mixed "A-B" tails can also commonly be found in particles that have the genotype either "A" or "B" (Streisinger, 1956a, b) These results indicate that tail proteins may be formed under the direct control of each of several phages present in the same cell, but the various proteins that are made are used more or less at random in the assembly of phage particles This is another consequence of the loss of identity of the phage particle during this vegetative phase and it may be re-emphasized by stating that the DNA of a single phage particle does not preside over the manufacture of its own body and tail protein, but DNA and coat manufacture probably take place in different sites in the cell, and

the assembly of these subunits is fairly random

At the end of the maturation process the cell bursts, releasing phage particles, and the burst size is characteristic of the particular type of cell and virus, the state of bacterial nutrition and other factors, and ranges from 30 to several hundred. The burst may be brought about by an enzyme made under the direction of the virus and, under abnormal conditions, cell lysis may occur even when no mature particles are formed. Bacterial cells can also be lysed by exposing them to large amounts of virus In this case, the viruses do not get into the cell, and the mechanism of lysis is poorly understood.

In recent years, the development of the subject of phage genetics (Hershey and Rotman, 1949, Hershey and Chase, 1951) has done more than any other aspect of virology to revolutionize concepts concerning the nature of viruses Besides developing the methods of virus inheritance it has shed light on the whole problem of virus replication in the cell and has now gone much farther in contributing to and extending classic genetics and establishing a close link between DNA and inheritance For some aspects of genetic work, phage has proved to be the most favorable material

In the course of phage multiplication in host cells, virus mutations appear spontaneously and at random, affecting a number of viral properties Mutations affecting host range and the plaque type are readily recognized and have been studied most fully. Many mutants are quite stable and breed true Two sublines of the same virus stock may be obtained by selection of mutants, which differ from each other in respect to a number of known characters When a single bacterium is infected with these two strains (marked "ABC" and "abc" for example), the burst will contain a variety of progeny Some will be identical with one or the other parent, but some will have various combinations of characters, part from each of the two parents (e.g., "Abc," "ABc," "aBC," etc). The latter are called recombinants, and their occurrence indicates a sexual process in the production of phage. In classic genetic terms the virus is haploid, i e., it contains one set of genes. It has a single chromosome. As in the recombination between linked genes by crossing over in higher forms, the frequency of phage recombination is an indication of how far apart two genes are on the phage chromosome The phage chromosome may be mapped by the frequency of recombination, and map distances are additive, as in classic genetics

In some other respects phage genetics is not classic. In a cross involving two factors, the number of two reciprocal recombinants in the yield from a single cell is often not equal If three suitably marked phages are introduced into a single cell, one finds progeny with markers from all three parents, indicating that the reproductive process involves a number of rounds of mating Such an experiment shows that the infection of a bacterium with two agents does not produce a cross, as in classic genetic work, but is instead a population experiment (Visconti and Delbruck, 1953) The DNA from infecting organisms goes into a pool in the host bacterium where it is replicated Apparently, the replication process requires the pairing of two chromosomal structures for the formation of each daughter replica If the two structures come from different parents, there is a "copy choice" mechanism which enables the daughter to begin replicating one chromosome and finish with the other chromosome, thus enabling recombinants to occur Once such a replica is formed, it too can act as the pattern for still other replicas As particles are finished or mature, these chromosomal structures are withdrawn from the mating pool and, once coated, they become inert in the infected cell and do not re-enter the mating pool.

Studies of temperate viruses and lysogeny are covered in connection with the discussion on latency and tumor viruses.

Multiplication of Animal Viruses

The development of knowledge and technic in the animal virus field reflects very closely the discovery and the use of new host material, and we shall review the history of this branch of virology very briefly from this viewpoint. All technics for virus detection depend in the first instance on the demonstration of a pathologic condition, caused by the agent Later, other methods of virus detection may be used. The difficulty of establish-

ing a virus infection in a new host species is often formidable, and progress is often slow.

In the early part of the century, the main host was the rabbit. Inoculations were made into the brain (herpes), onto the cornea (variola), or into the skin (vaccinia). Monkeys were used for poliomyelitis, measles and yellow fever. In such animals, a quantitative approach to virology was all but impossible. Attention was focused on lesions, both gross and microscopic, and the study and the search for inclusion bodies was one of the main approaches and was important in diagnosis.

In the early 1930's, the use of the mouse and especially the intracerebral route of inoculation brought great changes. This animal provided not only a medium for cultivation [illegible]

new viruses of uncertain host range, many of which came from mosquitoes. In addition, the mouse provided a much more practical method of virus quantitation than existed before. At about this time the domestic ferret, then [illegible] for dog distemper work, proved to [illegible]

was not an important means of isolating new [illegible]

worked out in this species. In the use of mice, death was frequently used as the criterion of determining whether or not infection had occurred. The practice of examining the infected organ or looking at infected cells microscopically fell into discard, and newer virologists often did not know what inclusion bodies looked like.

Almost concomitant with the introduction of the mouse was the beginning employment [illegible]

tended by Burnet (Beveridge and Burnet, 1946). The embryonated egg provided [illegible]

bacteria and contaminating viruses. The absence of antibody production in the embryo was still another advantage. The formation of pocks on the chorio-allantoic membrane by vaccinia, herpes and other viruses provided the earliest means of enumerating animal virus particles. Some agents killed the embryo and were detected in that fashion [illegible]

new agents were first isolated and detected by any chick embryo technic alone.

The use of the chick embryo was a step forward, though not an especially large one, in the direction of a more quantitative approach to virology. Technics such as de-embryonation were developed in which the chorio-allantoic membrane alone was used for virus growth, but virus quantitation was still essentially on a basis of using widely spaced dilutions (3- to 10-fold) in large numbers of eggs, and the inaccuracies of titration were large. One of the reasons for the ability of many viruses to grow in the chick embryo is because it is an embryonic tissue. In this connection, the use of baby mice (Dalldorf and Sickles, 1948) for the isolation of intestinal viruses is also an example of exploiting the remaining embryonic potentialities of the cells of the newborn. A large number of viruses were isolated from the intestinal tract by this technic, and this was the first of several examples of the discovery of innocuous or latent viruses in human beings in great numbers.

The currently common technic for virus study involves tissue culture. Actually, tissue culture of a sort had been in use for many years, but the Maitland culture usually involved the infection of pieces of tissue surviving in vitro. These pieces of tissue supported the growth of a great variety of viruses, but infection was not usually accompanied by gross changes, and such methods were not much more advantageous than in vivo technics.

Following World War II, there were striking technologic advances in the culture of tissue cells in vitro. A variety of cells were cultivated on glass, and a real revolution in virologic practice was started when Enders and collaborators (Weller et al., 1949, Robbins et al., 1951) demonstrated that a wide variety of human and monkey cells, grown in vitro, would not only support the growth of poliomyelitis virus but also developed gross morphologic changes as well. This had the immediate effect, for example, of changing

poliomyelitis from one of the most difficult to one of the most accessible viruses for study Furthermore, cells cultivated in vitro had a wider range of virus susceptibility than their in vivo counterparts, and a number of new and "difficult" viruses became available for study (herpes zoster, varicella, measles, adenoviruses) The new technic also revealed a wealth of new latent agents (in cells of cultured kidneys and tonsils), the presence of which had not been suspected previously However, perhaps the most important single consequence has been the development of new methods for the enumeration of virus particles, as reviewed below.

Progress in all branches of science is dependent to a large extent on the technics available, and an increase in the accuracy of virus quantitation is bound to have a very marked effect on the quality of research with viruses The most common method of animal virus titration in the past has been one involving inoculation of serial dilutions of virus, each into a large number of animals The end point in such titrations was taken as the dilution (either directly tested or interpolated) of virus which caused half of the injected animals to die or show symptoms This type of test is described in detail elsewhere in this volume, and it will suffice to say here that it is often a poor method of quantitation because it is very inaccurate It is very difficult to bring the titration error below 100 per cent, and it is usually higher Essentially, this means that any effect being studied by titration had to be correspondingly large

In the past few years, virus particle enumeration technics have been devised which involve the formation of plaques in vitro in a manner quite analogous to the technic used for bacterial viruses (Dulbecco, 1952) Advances in tissue culture technic make it possible to grow sheets of cells on glass. For this purpose, either pure line cancer cells (HeLa) or so-called normal cells, or mixtures of cell types from trypsin-digested organs (kidney, whole chick embryos, amniotic membrane, etc.) are used, the choice being influenced by the tropisms of the virus to be tested. Such cells can form a continuous solid sheet, firmly attached to the bottom of a petri dish The virus to be tested, suspended in a small fluid volume, is incubated with the cells long enough for the virus particles to attach The unadsorbed virus is washed off, and the cells are overlaid with nutrient agar. Upon incubation, the cells which have been infected will yield or excrete virus, and the agar limits the spread of the infection to the neighboring cells, which yield virus in turn. Eventually, a small circular area of necrosis appears A vital dye (neutral red) applied to the plate is absorbed by living cells and increases the contrast between infected and uninfected areas. This method allows one to count viable virus particles with a high degree of accuracy and reproducibility Plaques are started by a single virus particle, and this makes it possible to isolate pure line cultures readily

While each plaque starts originally with the infection of a cell by a single particle, nevertheless, all particles in a virus suspension do not start plaques The reasons for this are several Many of the particles in a virus preparation may be inactive or defective Active particles may be adsorbed to cells at sites where they cannot go on and infect (abortive adsorption). Active particles in the inoculum may fail, just by chance, to come in proper contact with susceptible host cells during the adsorptive period These factors, especially that of inactive virus, are discussed further below Older technics, such as pock formation on the chorio-allantoic membrane, are operationally similar to plaque formation, but practically only vaccinia and Rous sarcoma virus give very reproducible results The use of hemagglutination as a means of estimating virus quantitatively has been mentioned already

The problem of presenting a coherent picture of the adsorption, the penetration and the multiplication of animal viruses is complicated by incomplete knowledge of many species This difficulty is made more acute by the gross differences of many animal viruses from each other, so that the adequacy of the model approach may be correctly questioned However, the state of existing knowledge virtually forces the use of a model system at the present time For this purpose we shall use the myxovirus group, including influenza, Newcastle disease (NDV), mumps and fowl plague (FPV) as illustrative material. This group is fairly homogeneous, and a certain amount of generalization from data on one

virus is permissible within the group These agents have already been described to some extent in the section on virus hemagglutination.

Viruses of the myxo group are nearly round in shape and, in cut sections, they are from ... (Morgan et al 1956) in diam- ... ed out, ... prep- ... me in- ... entous ... fluenza ... out 0 9 ... 1957) ... chafer, 1957). None ... DNA They contain about 30 per cent lipid, 3 5 per cent carbohydrate, and the remainder is protein (Ada, 1957, Frisch-Niggemeyer and Hoyle, 1956, Schafer, 1957) The carbohydrate may be the same as host cell carbohydrate (Ada and Gottschalk, 1956) As noted above, all members of this group are able to agglutinate red cells and to destroy red cell receptors

The virus particles of influenza and FPV ... e ... h ... ether to remove the lipid fraction, which also contains carbohydrate and protein There- ... ill be found which ...

HA substance

The S antigen ■ small, 10 to 15 mμ. and from FPV it contains 10 to 15 per cent RNA That from influenza also contains the entire RNA of the intact particle The specificity of the S substance is that of a group antigen Antisera that react with influenza S substance give a strong cross-reaction with FPV S substance Serologic and other data indicate that the S substance in the intact particle is completely covered Antibody against the S substance has no neutralizing effect, for example, S substance is found outside of particles in the infected cell, and many infected cells produce an excess of a substance which is indistinguishable from the S extracted from the virus

The HA substance agglutinates red cells, and this carries the same specificity as the virus particle It reacts with neutralizing serum A specific HA serum does not react with S particles

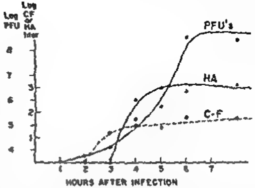


FIG 34 Graph after Breitenfeld and Schafer (1957), showing the rise in titer of complement-fixing (S) and hemagglutinating (HA) antigen in cells infected with fowl plague virus (FPV) The yield of plaque-forming particles is also shown Compare these results with those shown in Figures 35 and 36 where fluorescent antibody was used to detect these antigens.

There may be other antigens present in the virus, some of them possibly host antigens but so far they have not been differentiated

Adsorption and Penetration. How an animal virus gets into a cell is not yet known The membrane of an animal cell does not present the formidable barrier of the bacterial cell wall, and there is nothing about the morphology of animal viruses which suggests that the injection mechanism of phage is operative Viruses of this group, in general, adsorb rapidly to their host cells NDV has been shown to require electrolyte for attachment (Levine and Sagik, 1956) During the earliest phase of adsorption the virus is still accessible to antibody, and this initial phase ■ followed by penetration, which is a process that is highly temperature-dependent With penetra- ...

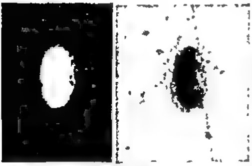
host cells has not been worked out in detail They are capable of infecting a variety of tissues in a variety of animals Cells in tissue culture are, in general, more susceptible to infection than those in the intact organism There is no doubt that the tropisms of these viruses and of animal viruses in general are much broader than those of bacterial viruses

2 hours (S antigen)

3 hours (H antigen)

FIGURE 35
(*Caption on facing page*)

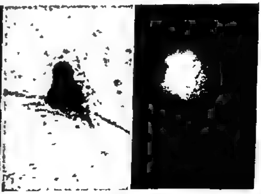
3 hours (S antigen)



3 hours (S antigen, Giemsa stain)

4 hours (H antigen)

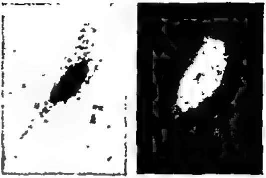
5 hours (S antigen)



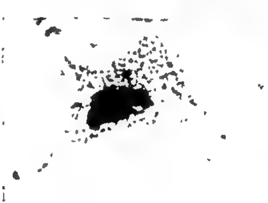
5 hours (H antigen, Giemsa stain)

FIGURE 35 (*on facing page*)
FIGURE 36 (*on this page*)

These are pictures of fibroblasts that were infected with fowl plague virus (FPV) and stained with fluorescent antibody after varying intervals Some were stained for S antigen and some for HA antigen. The S antigen was not present at 2 hours but was plentiful in the nuclei at 3 hours Even at 10 hours (Fig 36), the nucleus contained more antigen than the cytoplasm (Breitenfeld and Schafer, 1957). The HA antigen was absent at 3 hours but, when it appeared at 4 hours, the cytoplasmic concentration was already high The bright spots seen at 4, 5 and 6 hours are unexplained—they are extranuclear At 14 hours, the HA antigen can be seen to be concentrated in the periphery of the cell (see Figs 34 and 37)



6 hours (H antigen, Giemsa stain)



10 hours (H antigen, Giemsa stain)

10 hours (H antigen)

14 hours (S antigen)

14 hours (H antigen)

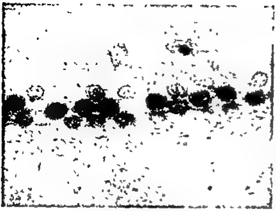


FIG 37. An electron microscope picture of a section through a chorio-allantoic cell infected with influenza virus The virus particles are organized units seen only in the cell wall The structure of the influenzal particles can be seen much better, and those at the lower limit of the membrane are more hazy in outline, suggesting incomplete maturation. (Morgan, et al, 1956, Structure and development of viruses observed in the electron microscope. III. Influenza virus J Exper Med *104*, 171-182)

Adsorption to cells is not always followed by penetration and infection In some cases, it seems that the virus has difficulty in going from the reversible to the irreversible stage of adsorption. This abortive adsorption may be more common with cells which are relatively resistant to infection

Penetration itself is not understood As stated in a previous section, the virus is adsorbed to receptor sites, and there is a virus enzyme available for lysing the site, but there is no direct evidence to indicate that such lysis actually does take place or is necessary. The number of attachment sites on a cell (if a red cell is used as a model) has been estimated as low as 300 (Sagik et al, 1954), though electron microscopy indicates a figure in the thousands (Isaacs, 1957) Whether all or only part of the virus goes into the cell is not known While the penetration of myxoviruses may proceed in a specific fashion, the infection of cells by defective (phenol-treated RNAase-sensitive) particles of poliomyelitis, for example (Colter et al, 1957), suggests that there may be some nonspecific method of virus entry.

The Eclipse Period. Following adsorption and penetration, there is a period when most of the adsorbed virus cannot be recovered as infective virus. At least 90 per cent becomes noninfective in the sense that it cannot be recovered from broken cells However, the recoverable active virus remains active and appears to be virus that has adsorbed to the cells in such a way that some but not all is elutable by RDE. Some is accessible to antibody and some not (Rubin et al, 1957) During the eclipse phase, the number of such particles may decrease somewhat, and it seems unlikely that they multiply. This small persistent active fraction and the disappearance of the majority of adsorbed virus is typical of a great many animal virus infections.

The important sequence of events during this latent period has been studied with both influenza and FPV, and the latter will be recounted in some detail If cells are infected with FPV (Breitenfeld and Schafer, 1957), there is a residual infective titer of about $10^{3.5}$ as shown in Figure 34

If aliquots of these infected cells are broken open at various intervals and examined for infective virus, S antigen (by complement fixation) and HA antigen (by hemagglutination), it is found that the S antigen appears first 3 hours after infection (Fig 34) and before there has been a significant rise in infective titer. Likewise, the HA titer goes up at 4 hours, just before infective virus appears Even more striking, however, is the examination of these cells with specific fluorescent antibody for the S and HA antigens in the infected cell, which corroborates the curves in Figure 34 and shows the location of the antigens being formed. From these results (Figs 35 and 36), it is seen that S antigen appears at 3 hours in the nucleus and spreads slowly to the cytoplasm However, the HA antigen appears at 4 hours and, from the beginning, is most prominent in the cytoplasm Even at 6 hours there is not much HA antigen found in the nucleus (Fig 36) Finally, the greatest concentration appears in the outer limiting membrane of the cell with the HA antigen at 14 hours

These studies are beautifully corroborated by electron photomicrographs of influenza virus infected cells (Morgan et al, 1956) Even by careful search no finished, mature,

fully coated particles are seen anywhere in the infected cell nucleus or cytoplasm Mature particles are seen only in the outermost layer of the cell (Fig 37), where they are found in abundance and appear in such form that one is tempted to say that they reach maturation almost at the moment of reaching the outer membrane

More evidence on this point can be seen from examining infected cells of the chorio-allantoic membrane in de-embryonated eggs Such cells, after beginning to discharge virus, continue to do so for many hours If at any time the cells are opened, noninfective hemagglutinin may be found inside which may be the precursor material for mature virus The life of the mature particle in the cell is short, probably only 30 minutes (Henle et al, 1956) The virus in the outer layer can be freed from the cell at any time by the action of RDE This suggests that the virus enzyme plays a role in extrusion

From the foregoing facts, the following picture emerges Virus which is properly adsorbed penetrates the cell, where it goes into a noninfective stage, the details of which are not known The first sign of virus multiplication then appears in the cell nucleus, where the RNA antigen is being synthesized This material then goes out into the cytoplasm where the HA antigen is being formed, and the final process of assembly is the covering of the S antigen with HA and lipid material This appears to occur just at the limiting membrane of the cell and extrusion of the particles takes place without gross or even microscopic evidence of damage to the cells, which continue to produce virus over long periods of time Other evidence consistent with this view is the action (LeClerc, 1956) of ribonuclease in preventing virus multiplication in cells at one stage, although they reach a refractory state late in production, indicating the appearance of complete particles Evidence of phenotypic mixing, the production of coats which come from two different parents, has been obtained with these viruses, and this is consistent with a picture of the separate production of S and HA antigens This evidence is reviewed with studies of multiple infection

Formation of Filaments Some strains of influenza virus, especially those that are examined shortly after isolation from human infections, contain large numbers of long filaments mixed with the usual spheres The filaments are about the width of virus particles (Fig 38) but often several hundred times as long They are made up, at least in part, of specific virus material Red cells stick to them at any point When the filaments are mechanically broken up, the HA titer of the suspension rises, but the infective titer does not These long, somewhat brittle rods contain perhaps one infectious particle The internal structure (Morgan et al, 1956) is not like that of influenza virus, and the rods are lysed by agents which are destructive to the host cell membrane but not to normal virus particles, suggesting that the filaments may include normal cell constituents (Burnet, 1956). There are similar filaments, which

FIG 38 Showing influenza filaments coming from the wall of an infected cell They are devoid of internal structure (Morgan, et al, 1956, Structure and development of viruses observed in the electron microscope III Influenza virus, J Exper Med 104, 171-182)

have no HA activity, extruded from normal cell membranes. It seems possible that there is some abnormal mechanism of virus extrusion at work, possibly in an area of the cell where no S substance is present, in which both host and virus elements get involved

Formation of Noninfectious Virus. It seems to be quite a common phenomenon among viruses that a fairly large proportion of the virus formed is inactive It is also likely that a number of different phenomena are responsible for this lack of activity Some of the various types of inactive influenza virus may be listed as follows:

1. Noninfective virus in normal yields In virus prepared under what may be considered as optimal conditions, a large proportion of the seemingly normal particles do not infect By comparing the infectivity of influenza virus for eggs, or its ability to form plaques or to form red cell dimers under proper conditions, with a particle count as determined by electron microscopy (virus particles absorbed to red cell ghosts), it is found that only about 10 per cent of the influenza hemagglutinating particles infect (Isaacs, 1957)

A. Part of this noninfective virus may actually be intact virus which fails to infect because it remains unadsorbed, perhaps due to lack of effective contact between virus and cells

B Part of the inactivity may be due to abortive adsorption. This involves adsorption to sites from which infection cannot proceed, or possibly adsorption to cells which are not competent to produce virus.

C The noninfective virus may be defective virus, and it may be defective because of faults in assembly of parts or some of the subunits may in themselves be defective Some of these possibilities will be considered under (2) and (3).

D. Another possibility is that the virus may have been thermally inactivated after formation. In the case of allantoic fluid virus, some of the earlier particles produced may rest in the allantoic sac at 37° for long periods of time and the half-life of a particle at this temperature is short (Horsfall, 1955).

2. Noninfectious virus from yields where the input multiplicity was very high When cells are inoculated with certain influenza strains under conditions where the infecting multiplicity is high (2 or more per cell), the yield in terms of hemagglutinin may be almost normal, but the number of infective particles (allantoic sac) may be markedly depressed (von Magnus, 1954). The number of infective particles per HA unit in "normal" virus is usually 10^6 or slightly more, while the yield after a large inoculum may have only 10^2 or less infective units per HA unit The effect is increased sometimes through several egg-to-egg passages with large inocula Some strains give this inactive virus more readily than others. When these yields of low infectivity are examined in the electron microscope, the number of particles may be nearly normal, but many particles appear flattened The particles are less dense than normal ones, possibly because they contain a higher proportion of lipid, and the amount of RNA per particle for influenza may be only 30 per cent of normal (Ada, 1957). There is some evidence that the so-called inactive particles do infect but give rise, in turn, to defective particles. If cells are multiply infected with inactive virus (UV) plus a single particle of normal virus, the yield will have a low infectivity/HA ratio It is possible that multiple infection of the cell induces a very disordered virus production process In interpreting these results, one must be cautious to eliminate other ancillary causes of inactivation, such as heat NDV does not produce this effect

3 Inactive virus from special hosts If influenza virus (ordinary strain) is inoculated into the brains of mice, a hemagglutinin is produced (Schlesinger, 1950), but infection is limited to a single cycle, and the "virus" produced is inactive It apparently agglutinates normally but does not infect; hence, the course in the brain is limited to a single cycle. Infection of HeLa cells produces a similar reaction (Henle et al., 1955) This is like the previous type of inactive virus except that it appears to be completely noninfective In the electron microscope the particle appears to be flattened Some host cells are incapable of producing perfect virus particles, for reasons unknown

Almost all animal viruses that have been investigated show a discrepancy, often a large one, between the total particle count and the count of infective particles With bacterial

viruses, the discrepancy is very small. It is tempting to speculate that the process of assembly from subunits is one which can take place more efficiently in the small compass of a bacterial cell than in the much larger volume occupied by most mammalian cells. Whatever advantages the animal virus gains by having subunits manufactured in different pools and then assembled is evidently done at some, or perhaps a large, sacrifice in efficiency so that many defective units are produced or subunits remain unused and wasted. It has been suggested that, since the assembly process is something more typically viral than the other synthetic processes going on in an infected cell, derangement of the assembly mechanism may be a fruitful point of attack for chemotherapy, for example (Luria 1958).

Genetics of Myxoviruses. Animal viruses appear to contain either DNA or RNA but probably not both. The myxoviruses are RNA viruses and therefore are of special interest because, thus far, the mechanics of inheritance by means of RNA are not known. Influenza contains about 1 per cent and FPV about 3 per cent RNA and, in each case, all of this appears to be located within the particle as part of the S antigen. With high input multiplicities of virus, the yield contains less RNA per particle (cf. incomplete virus, above). The base ratios in the RNA differ from strain to strain, and there is also some unconfirmed indication that the base ratios may change in the same virus, depending on the host species in which it grows (Ada, 1957). Electron photomicrographs of myxoviruses after preliminary treatment with trypsin and other reagents can be stained with phosphotungstic acid to show a many-stranded ring structure, which may possibly be RNA (Valentine and Isaacs, 1957a, b).

The present knowledge of myxovirus genetics may be summarized briefly by stating that it is in a very primitive state. This is due, at least in part, to the fact that only very crude methods of examination have been used. Most of the reported work has been done with influenza A. A number of naturally occurring strains of this organism differ from each other in terms of antigenic pattern, virulence, heat stability of hemagglutinin and other characters. When cells are infected, each with the two virus types, the yield from this infection may contain some particles in which the original characters from two different parents appear (recombinants). The best example (Burnet and Lind, 1951) consists of a cross between two different strains which differ by serotype (W and M) and in virulence for the mouse (intracerebrally) (V and A). From a mixed infection of W-V and M-A can be derived the parent types and also W-A, which is recombinant. Strain M-V occurs rarely. The W-A strains appear to be fairly stable and retain their new characters on passage. It is also possible to recover characters from heat or UV inactivated virus (Burnet and Lind, 1954; Gotlieb and Hirst, 1956). If strain W-V is inactivated by UV and inoculated into eggs with active M-A strain M-V can be recovered quite readily. Apparently, the active virus can rescue a character (virulence) from the inactive agent.

A number of other characters have been used in recombination tests, but they will not be reviewed here, since they do not as yet lead to any clear-cut interpretation of RNA genetics. What is presently lacking is quantitative data on frequency of recombination, on yields from single cells, on linkage and other parameters. There is no doubt that exchange of characters does occur with some viruses, but the mechanism of exchange is not clear. Similar preliminary findings have been reported with herpes and with psittacosis and vaccinia.

Phenotypic Mixing. In the section on bacterial viruses, the phenomenon of phenotypic mixing was discussed. Double infection of a bacterial cell with two phage strains resulted in the formation of a tail which was like type "A" (for example) in a virus which was genotypically "B" and vice versa. Tails were also found which contained mixed antigens "A" and "B." A formally analogous situation is found with influenza virus. If influenza strains, type A and B, are used to infect a single cell, it can be shown that as many as 85 per cent of the progeny of this infection will have both A and B antigens on the virus surface (Gotlieb and Hirst, 1954). Nevertheless, the virus particles are all either A or B genetically. This is another example of the result of separate production and assembly of virus subunits. When one realizes

have no HA activity, extruded from normal cell membranes. It seems possible that there is some abnormal mechanism of virus extrusion at work, possibly in an area of the cell where no S substance is present, in which both host and virus elements get involved

Formation of Noninfectious Virus. It seems to be quite a common phenomenon among viruses that a fairly large proportion of the virus formed is inactive It is also likely that a number of different phenomena are responsible for this lack of activity. Some of the various types of inactive influenza virus may be listed as follows.

1. *Noninfective virus in normal yields.* In virus prepared under what may be considered as optimal conditions, a large proportion of the seemingly normal particles do not infect. By comparing the infectivity of influenza virus for eggs, or its ability to form plaques or to form red cell dimers under proper conditions, with a particle count as determined by electron microscopy (virus particles absorbed to red cell ghosts), it is found that only about 10 per cent of the influenza hemagglutinating particles infect (Isaacs, 1957)

A. Part of this noninfective virus may actually be intact virus which fails to infect because it remains unadsorbed, perhaps due to lack of effective contact between virus and cells.

B. Part of the inactivity may be due to abortive adsorption This involves adsorption to sites from which infection cannot proceed, or possibly adsorption to cells which are not competent to produce virus

C. The noninfective virus may be defective virus, and it may be defective because of faults in assembly of parts or some of the subunits may in themselves be defective. Some of these possibilities will be considered under (2) and (3)

D. Another possibility is that the virus may have been thermally inactivated after formation. In the case of allantoic fluid virus, some of the earlier particles produced may rest in the allantoic sac at 37° for long periods of time and the half-life of a particle at this temperature is short (Horsfall, 1955).

2. *Noninfectious virus from yields where the input multiplicity was very high.* When cells are inoculated with certain influenza strains under conditions where the infecting multiplicity is high (2 or more per cell), the yield in terms of hemagglutinin may be almost normal, but the number of infective particles (allantoic sac) may be markedly depressed (von Magnus, 1954). The number of infective particles per HA unit in "normal" virus is usually 10^6 or slightly more, while the yield after a large inoculum may have only 10^2 or less infective units per HA unit. The effect is increased sometimes through several egg-to-egg passages with large inocula. Some strains give this inactive virus more readily than others. When these yields of low infectivity are examined in the electron microscope, the number of particles may be nearly normal, but many particles appear flattened The particles are less dense than normal ones, possibly because they contain a higher proportion of lipid, and the amount of RNA per particle for influenza may be only 50 per cent of normal (Ada, 1957). There is some evidence that the so-called inactive particles do infect but give rise, in turn, to defective particles If cells are multiply infected with inactive virus (UV) plus a single particle of normal virus, the yield will have a low infectivity/HA ratio It is possible that multiple infection of the cell induces a very disordered virus production process. In interpreting these results, one must be cautious to eliminate other ancillary causes of inactivation, such as heat. NDV does not produce this effect.

3. *Inactive virus from special hosts* If influenza virus (ordinary strain) is inoculated into the brains of mice, a hemagglutinin is produced (Schlesinger, 1950), but infection is limited to a single cycle, and the "virus" produced is inactive It apparently agglutinates normally but does not infect; hence, the course in the brain is limited to a single cycle Infection of HeLa cells produces a similar reaction (Henle et al, 1955). This is like the previous type of inactive virus except that it appears to be completely noninfective In the electron microscope the particle appears to be flattened Some host cells are incapable of producing perfect virus particles, for reasons unknown

Almost all animal viruses that have been investigated show a discrepancy, often a large one, between the total particle count and the count of infective particles. With bacterial

viruses, the discrepancy is very small. It is tempting to speculate that the process of assembly from subunits is one which can take place more efficiently in the small compass of a bacterial cell than in the much larger volume occupied by most mammalian cells. Whatever advantages the animal virus gains by having subunits manufactured in different pools and then assembled is evidently done at some, or perhaps a large, sacrifice in efficiency so that many defective units are produced or subunits remain unused and wasted. It has been suggested that, since the assembly process is something more typically viral than the other synthetic processes going on in an infected cell, derangement of the assembly mechanism may be a fruitful point of attack for chemotherapy; for example (Luria, 1958).

Genetics of Myxoviruses. Animal viruses appear to contain either DNA or RNA but probably not both. The myxoviruses are RNA viruses and therefore are of special interest because, thus far, the mechanics of inheritance by means of RNA are not known. Influenza contains about 1 per cent and FPV about 3 per cent RNA and, in each case, all of this appears to be located within the particle as part of the S antigen. With high input multiplicities of virus, the yield contains less RNA per particle (cf. incomplete virus, above). The base ratios in the RNA differ from strain to strain, and there is also some unconfirmed indication that the base ratios may change in the same virus, depending on the host species in which it grows (Ada, 1957). Electron photomicrographs of myxoviruses after preliminary treatment with trypsin and other reagents can be stained with phosphotungstic acid to show a many-stranded ring structure, which may possibly be RNA (Valentine and Isaacs, 1957a, b).

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that the genetic material of the virus is produced in the nucleus and the type-specific material (hemagglutinin) is produced in the cytoplasm and probably applied to the core just before extrusion, it becomes clear why the outer and the inner parts of the virus do not coincide in terms of origin It also illustrates the fact that the coating pools of different viruses can get mixed in the cell and both will fit into the same virus coat The same situation is encountered with poliomyelitis virus types I and II (Sprunt et al, 1955, Ledinko, unpublished) where mixed infection of HeLa cells results in virus which can be neutralized by either type I or type II serum

The Behavior of Other Animal Viruses in Mammalian Cells. It will be useful to consider briefly a few other viruses in somewhat the same way that the myxoviruses have been covered

Polioviruses. This is an interesting group of viruses Until their ability to grow in many kinds of tissue culture was demonstrated, they could be handled in the laboratory in only the most restricted manner The virus is a small particle, about 30 mμ in diameter, round in shape and very stable in the extracellular form It can be extracted with ether without loss of titer, probably because it contains no lipid, only protein and RNA. RNA is present to the extent of 25 per cent When purified, the virus can be crystallized (Schwerdt and Schaffer, 1956) In intact animals, the strains freshly isolated from human beings will multiply satisfactorily only in primates, though some strains have been adapted to the intracerebral route in mice and to chick embryos. [illegible] stream In tissue culture preparations, it will infect a variety of cells of primates, such as kidney epithelium, muscle or embryonic and cancerous tissues It multiplies poorly in primate kidney cells in the intact animals (Kaplan, 1955)

Much of the recent investigation of the virus has been carried out in tissue culture, either of monkey kidney or of HeLa cells Adsorption of the virus to these cells is fairly rapid and complete and requires calcium (Bachtold et al., 1957). Nothing is known about sites of adsorption on the cells or about penetration. Once inside the cell, multiplication is rapid, and virus is excreted after 3 to 6 hours, depending on the host cell and conditions of cultivation Cells that begin to excrete virus usually die within a short period of time (Lwoff et al, 1955), disgorging the cell contents Each cell may produce as many as 20,000 particles but, for a virus the size of poliomyelitis, this constitutes less than 2 per cent of the total cell mass The relatively small proportion of total cell metabolism which is due to virus synthesis is also reflected in the fact that HeLa cells can synthesize virus very well when they are in a nutrient medium consisting of (Eagle and Habel, 1956) electrolyte and glutamine. Since this is insufficient for the nutrition of the cell alone, the virus is being synthesized out of cell reserves

When poliomyelitis virus is adsorbed to cell monolayers overlaid with nutrient agar, cell lysis also occurs, and the plaques (compared with NDV or influenza) are large and grow rapidly, since the virus is small and diffuses rapidly Only 3 to 4 per cent at most of the particles in a suspension (from monkey kidney cells) form plaques Again, the reasons are not clear, but the occurrence of imperfect particles is one possibility. By means of differential centrifugation, the virus population has been separated into two fractions (Le Bouvier et al, 1957). One fraction is infectious, contains 25 per cent RNA and gives a distinct band in an agar diffusion test against immune sera The other fraction is not infectious, contains protein but little RNA, and gives a different band in the immunologic test It is not possible to say without further evidence whether the second fraction is a virus precursor which had not yet been incorporated into finished particles or whether it was defectively assembled virus in which RNA was omitted or in which the antigens were defective

There are 3 serologic types of poliomyelitis virus, unrelated by neutralization tests However, if cells are infected with both type I and type II virus, the yield will contain a majority of particles which can be neutralized by both type I and type II antisera. Such particles contain a mixture of I and II antigens, and since, genotypically, the particles seem to be either I or II (Ledinko, unpub-

lished), a separate formation of genetic and coat material seems to be very likely

This point of view is also supported by experiments in which poliomyelitis particles are damaged by high concentrations of phenol. This treatment renders the RNA in the particle accessible to RNAase action, yet the particles are still able to infect, albeit with a low efficiency (Colter et al, 1957) These experiments suggest, as do the similar ones with tobacco mosaic virus, that the essential material for carrying the information necessary for virus synthesis does not reside in the protein coat but rather in other elements, such as RNA

A number of mutants of poliomyelitis virus have been described, especially since the development of the plaque technic Some of these, such as a strain adapted to growth in the chick embryo are doubtless multistage mutants isolated by a prolonged selective process Mutants arise spontaneously and maintain their character on subpassage Mutations involving changes in plaque type, heat stability, resistance to normal serum inhibition, neurotropism and a host of other characters, have been described Where the back-mutation rate has been measured, it was found to be of a similar order of magnitude as that found with DNA viruses The behavior of mutants suggests that the virus is haploid, and there are no obvious special peculiarities of RNA mutants Recombination with poliomyelitis has not yet been accomplished

Vaccinia Virus. In many ways this virus is very different from poliomyelitis It is very large, about 200-300 mμ in diameter It is very complex chemically, containing proteins, lipids, carbohydrate and DNA At least three separate soluble fractions have been described (L, S and HA) which can be separated from the virus particle without loss of viability By electron microscopy it is also complex, with a double outer membrane and a complicated center like a nucleus (Fig 39) (Morgan et al, 1954) While mainly dermatropic in its natural host, it has been adapted to a variety of animal hosts and grows well in tissue culture Its attachment mechanism to cells is not understood, and the virus is neutralized poorly with immune sera When this virus enters a cell, there is a loss of infective titer, though with some hosts this is slight The site of virus synthesis is not known and, by electron microscopy, the particles appear at first in a homogeneous matrix which is just outside the cell nucleus This matrix contains a lot of DNA, and the particles appear in this area by what may be stepwise maturation (Gaylord and Melnick, 1953) When first visible as particles in the cell, they are surrounded by a single membrane After extrusion from the cell, the outer membrane is double (Morgan et al, 1954) The areas of virus concentration are described in classic pathology as inclusions

FIG 39 Two particles of vaccinia virus—one shows the double outer membrane very well, and the other shows the light nucleuslike body and the internal structure (Morgan, et al, 1954, Structure and development of viruses observed in the electron microscope II Vaccinia and fowl pox viruses J Exper Med *100*, 301-310)

Vaccinia virus grows on the chorio-allantoic membrane of eggs, where it forms lesions which are necrotic but also show some proliferation In this and other respects, they are distantly related to tumor-inciting agents, such as the virus of rabbit fibroma The virus also forms proliferative lesions in tissue culture Laboratory strains differ from each other in a number of characters, and with mixed infection of single cells recombinations of characters occur which are stable on passage (Fenner, 1958)

Tests for the infectivity of vaccinia preparations show that a variable proportion of the population will not, or at least does not, infect. The reasons for this are not clear.

Adenoviruses The viruses of this group have been discovered recently, and they are difficult to handle, even with tissue culture methods This is an example of a small DNA virus Its size is 60 mμ Little is known about its mode of adsorption to the cell, but multiplication appears to occur solely in the nucleus The virus appears in the nucleus in large crystalline arrays which give a positive *Feulgen test for DNA (Bloch et al., 1957)* With time, the crystals break up, and the viruses become dispersed in the cytoplasm Inclusions of a crystalline protein are found in the virus crystals (Morgan et al, 1957).

Very few of the statements made in the previous section about myxoviruses can be used as a basis for generalities about animal viruses because of lack of direct information In respect to virus composition, there are at least two major divisions: viruses that contain no lipid and those that do, and those which contain RNA or DNA The viruses which contain no lipid are, in general, small and are resistant to the action of ether. Members of this group include poliomyelitis, Coxsackie viruses, adenoviruses, etc (Andrewes and Horstman, 1949) All animal viruses appear to contain either RNA or DNA None is known to contain both The myxo agents and poliomyelitis are examples of RNA; adenoviruses, vaccinia and probably herpes are examples of DNA viruses

Very little can be said about receptors or attachment mechanisms of any viruses except the myxo group After penetration, an eclipse period is found for nearly every virus that has been tested adequately, including Rous sarcoma virus (Prince, 1958) Growth curves usually show that something of the order of 90 per cent or more of the absorbed virus disappears. In no case is the disappearance complete, and the beginning of new synthesis is taken as the time when there is an increase in infective titer. The eclipse or latent phase varies from a few hours to 10 or more hours, depending largely on the virus, conditions of growth, etc. The mode of release is frequently obscured by rapid re-adsorption.

Evidence for the production of several virus constituents in separate sites of the cell is lacking for most viruses. The early appearance of S substance from myxoviruses in the nucleus has been mentioned. Herpes virus antigen also appears first in the nucleus and later in the cytoplasm (Lebrun, 1956) Adenoviruses appear in the nucleus also For the other viruses, the synthesis of particles from separately produced constituents in the cell is not established and it is still possible that the eclipse phase for psittacosis, for example, may be a case of masking rather than dissolution

Changes in the Host Cell. *No animal* virus has been found which has an intrinsic metabolism of its own, and it is generally assumed that virus synthesis inside the host cell is accomplished with energy mobilized by the enzyme systems of the host Inhibitors of energy production such as cyanide, or an uncoupler of phosphorylation like 2-4 dinitrophenol, are generally able to block virus synthesis (Eaton, 1952) This is taken to mean that virus synthesis is dependent on the Kreb's cycle enzymes, for example. This approach to control of virus multiplication is elaborated in Chapter 6.

The morphologic changes in infected cells are also quite varied, and they have long been the object of intensive study by pathologists Unfortunately, much of the pathology which occurs is not very specific in terms of the processes which are going on Disintegration of the nucleus, disintegration of the cell mem-

croscopy, it has not been feasible to reconstruct a logical series of events leading up to the demise of the cell. Inclusion bodies are

the only fairly certain morphologic sign of virus infection

Inclusion bodies are areas staining characteristically within cells which are the result of infection. The inclusions may be either in the cytoplasm or the nucleus. They may be made up of virus particles, or they may be scars of former infection, and the latter case is usually true of intranuclear inclusions. Intranuclear inclusions have been classified as type A or B by Cowdry, on the basis of severity, the latter being milder.

The inclusions which have received the most detailed descriptions are those of the larger viruses, like psittacosis or vaccinia. Intracytoplasmic inclusions are found in a number of human virus infections such as psittacosis, variola, rabies and the encephalitides. In the case of psittacosis (Bedson and Bland, 1932), it was found that a large amorphous mass first appeared in the cell cytoplasm. After some time, formed elements appeared in it and developed in a succession of forms not unlike that of malarial parasites. Micromanipulation and micropipetting of psittacosis and fowlpox inclusions have demonstrated that they contain the infective agent. In fact, such inclusions probably are made up largely of virus particles and, in some instances, they appear to be surrounded by a membrane.

Intranuclear inclusions have been studied in less detail than the cytoplasmic ones. In some infections such as herpes simplex, the infection leaves a nuclear scar which has been shown by fluorescent antibody (Lebrun, 1956) to be nonspecific, i.e., to contain no virus antigen. It is relatively easy to stimulate the formation of intranuclear inclusions by rather general types of cell injury, not involving viruses. On the other hand, certain infections such as those with adenoviruses produce intranuclear inclusions which are virus crystals. Infections in which intranuclear inclusions occur include herpes zoster, herpes simplex, varicella and pseudorabies of swine. B type inclusions occur in yellow fever and in Rift Valley fever. Occasionally the presence of inclusion bodies is used as evidence for virus infection. Rabies is a good example of the use of inclusions diagnostically.

Multiplication of Plant Viruses

Viruses are perhaps the most common type of agent causing disease in plants. Plant viruses are known only among angiosperms or flowering plants, but perhaps this is only because they have been studied the most thoroughly. The best-studied examples are the infections of economically important plants.

Viruses may be introduced into plants in a variety of ways: by insects, wind, soil, contact and grafting. Dodder, which is a plant parasite, can be used to transfer infection from one plant to another by having it parasitize two plants at once. Once a plant is infected, the infection may be carried on through vegetative propagation of the plant (as in potatoes), or the virus may pass to other generations through the seed or rarely through pollen.

The virus usually spreads rapidly after infection through the whole plant via extracellular fluid. An exception occurs when the site of infection becomes necrotic so rapidly that further extension of the infection stops, trapping the virus. This is called "hypersensitiveness." Sometimes plants that are infected recover in the sense that late-appearing shoots look normal although they contain virus. The reason for this tolerance is not clear. The local necrosis reaction of hypersensitivity furnishes the basis for a pock method of counting infectious particles. Infection of a plant with one virus which in itself may be asymptomatic may produce immunity (interference) against another lethal strain.

Plant viruses offer extremely favorable conditions for some types of study and very great difficulty for others. As in the other virus groups, the principal attention of investigators has been focused on a single virus, tobacco mosaic virus (TMV). There are a number of hosts for this virus but, in actual practice, tobacco plants are usually used either in entirety or as detached leaves.

The virus is a rod which is about 300 mμ long on the average and 15 mμ wide, and consists of an inner helix of RNA and an outer coat of protein. The virus is titrated by rubbing on *N. glutinosa* leaves. Wherever hair cells are properly injured by this treatment, a virus particle may enter a cell and start an infection. Because of hypersensitivity, the infection becomes localized, and each site can be counted as a plaque. Because of a number of variables, it is extremely difficult to get reproducible counts by this method, and usually determinations are made in terms of comparing two preparations, inoculated on oppo-

site halves of the same leaves. In addition, the efficiency of infection is very low, and it may require as many as 10^6 particles of virus to initiate a single necrotic lesion This difficulty in infecting cells with small amounts of virus and the absence of any specific mechanism for attachment of viruses to host cells is characteristic of plant viruses The use of such large amounts of virus on the leaf for infection precludes any very decisive study of early infection in the leaf. There is no method of infecting and studying single cells To counteract these disadvantages is the relatively simple structure of plant viruses, and the possibility of getting very large amounts of highly purified material, since the virus can be crystallized readily TMV makes up 10 per cent or more of the dry weight of the plant, and a single cell may yield 60 million particles

The simple structure of the virus has been mentioned already It is a long protein tube, and the RNA is buried within it The virus contains 5 6 per cent RNA, which is not accessible to RNAase in the native virus form. Crystallographically, the virus is made up of small (27 mμ) units of length which are repeated throughout the entire length In a preparation from a plant, the particles are of a wide variety of lengths, although this may be due to manipulation, and there is much evidence that 300 mμ is the native size and the infective size.

This brings up the problem, as with other viruses, of the noninfective particles, and again the answer is not clear It may take 10^5 to 10^6 particles to produce a plaque The methods of inoculation are crude, and a great deal of wastage cannot be eliminated The occurrence of precursors or incomplete virus will be taken up later It is also possible that much virus may be inactivated by breaking the rods at less than the optimal length

TMV is made up of RNA (5 6%) and protein The two may be readily separated from each other. Under proper conditions, the virus protein has a tendency to reaggregate in the form of rods—rods which are of quite variable length and do not contain any RNA (Schramm, 1947). When protein and RNA are mixed under similar conditions, the RNA forms a core around which a protein coat assembles. This appears very much like the original particles, and these reformed particles are infective, sometimes to 30 per cent of the original value (Fraenkel-Conrat, 1957). If the RNA from one virus is coated with the protein from another virus, the resulting agent will give rise to virus which corresponds to the RNA present and not to the protein RNA carries the genetic specificity (Fraenkel-Conrat, 1956, Fraenkel-Conrat and Williams, 1955).

Finally, RNA itself is infectious (Gierer and Schramm, 1956), again with an efficiency of about 1 per cent of the virus from which it came In this form, the virus was not very stable. It was easily damaged by heat, and the RNA was accessible to RNAase which destroys it.

In view of these results on the primacy of RNA in infection, it is not surprising that it is possible to bring about extensive changes in the protein coat of virus particles (blocking amino groups with iodine for example) without seriously altering the ability of the virus to infect (Stanley, 1943) The most likely role for the protein coat is that of a protector for the RNA.

The course of virus multiplication in infected plants has been studied extensively, but technical difficulties are great It is not known, for example, whether there is an eclipse period after virus infection, because there is such a tremendous excess of virus left on the leaf after inoculation There is also the difficulty that one must measure the average reaction of a great number of cells If the leaves are infiltrated with RNAase just before or just after infection with TMV, infection is completely inhibited, suggesting an early change in the virus after inoculation (Hamers-Casterman and Jeener, 1957) By 2 hours, the virus is RNAase-resistant On the other hand, if infected leaves are irradiated with ultraviolet light and the effect on the number of infective centers is determined, it will be found that the sensitivity of virus after entry into the leaf is the same as that of free virus (Siegel and Wildman, 1956) This condition lasts for about 2 hours after infection, after which the infective centers become progressively more resistant to inactivation and, after 5 hours, the inactivation curve becomes multiple hit. These changes are not easy to interpret but suggest that the virus

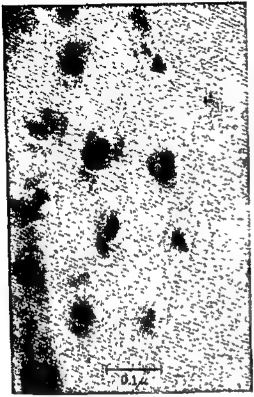

FIG 40 Portion of polyhedron of *B mori*, showing single viral particles included in the crystalline matrix (Morgan, C, Bergold, G H, Moore, D H, and Rose, H M, 1955, The macromolecular paracrystalline lattice of insect viral polyhedral bodies demonstrated in ultrathin sections examined in the electron microscope, J Biophys & Biochem Cytol *1*, 187-190)

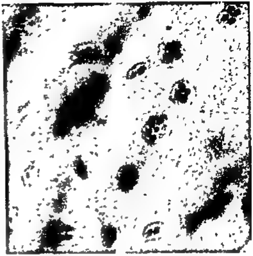

FIG 41 Portion of polyhedron of *P dispar*, showing characteristic bundles of rod-shaped viral particles inclosed by limiting membrane (Morgan, C, Bergold, G H, Moore, D H, and Rose, H. M, 1955, The macromolecular paracrystalline lattice of insect viral polyhedral bodies demonstrated in ultrathin sections examined in the electron microscope, J Biophys & Biochem Cytol *1*, 187-190)

goes through definite stages in which it is sheltered to different degrees from a UV effect

As with other viruses, the substance of TMV seems to be synthesized in the plant from small molecular weight materials assimilated by the plant after infection (Meneghini and Delwiche, 1951) Once made, the particles are stable with little or no turnover However, excess RNA accumulates in infected plants beyond what can be found in the virus (Basler and Commoner, 1956) Also, there are several RNA-free proteins found in the tissues of infected plants, and these consist largely of virus protein in various states of aggregation Some of this virus protein in the plant may be virus precursor protein, but some may have become aggregated without RNA to form noninfectious products The RNA itself is not highly regular in composition, as reflected in a variable base ratio for viruses grown under various conditions (Commoner and Basler, 1956)

Mutations in plant viruses are not uncommon, and there are numerous examples which seem to be stable mutants Recombination experiments at present are inconclusive The feeding of thiouracil to infected plants results in a large incorporation into TMV nucleic acid as a replacement of uracil (Jeener, 1956) Such virus has diminished infectivity

Persistent infection of plants without symptoms is a common event with plant viruses, and this is dealt with under the section on latency

VIRUS DISEASES OF INSECTS

Virus infections of insects can be grouped in 4 classes (1) agents that cause disease

and are lethal for insects, (2) agents that are pathogenic for vertebrates but also multiply in insects (or acaridae), (3) agents that cause diseases of plants but also multiply in insects and (4) an agent which produces CO_2 sensitivity in Drosophila. In the first 3 groups especially it is very difficult to perform adequate quantitative titrations of the viruses, and as a result knowledge of these agents is very restricted and it is summarized briefly.

Agents Causing Disease Exclusively in Insects. The viruses multiply extensively during the larval stage and, in the case of the Crane fly (Williams and Smith, 1957), come to make up 25 per cent of the weight of the larvae Many larval infections are accompanied by the production of large crystalline bodies which are made up of nonviral, nonlarval protein. Sometimes these are in the form of large polyhedra, sometimes small amorphous-shaped capsules Both forms are really protein crystals. The virus particles themselves are frequently caught up in these crystalline masses (Figs 40 and 41) where, by electron microscopy, they appear to be in a wide array of developmental stages (Bergold, 1953) In other diseases, the viruses are surrounded by a large, alkali-soluble capsule and are often surrounded by a membrane The virus particles contain DNA as the principal and probably sole nucleic acid The virus from the sawfly crystallizes readily

One further feature of these viruses which requires comment is the occurrence of latent infections In order to explain the sudden outbreaks of the disease in laboratory colonies, it is necessary to postulate that latent infection occurs over many generations in insects. Because of this and other features, these viruses are of great general interest, but the lack of a really good titration system is, at present, a serious handicap in studying them.

Agents Infecting Plants or Warm-Blooded Animals as Well as Insects. A number of viruses are known which attack man (cf. Arbor viruses, Chap 12) and are also capable of multiplying in arthropods, usually in mosquitoes among the insects and in ticks or mites among the acaridae. Very little quantitative work has been done with the infection in these arthropods, but it is known, for example, that yellow fever virus multiplies in the salivary gland and the stomach of *Aëdes egypti* mosquitoes, and there is some evidence for the transoverian transmission of other viruses in ticks. In fact, wild caught mosquitoes have been a good source of "orphan viruses," some of which have been shown later to cause infection in man (West Nile virus) or in arthropods

Several plant viruses (Black, 1953) have now been shown to multiply in certain diptera, and this has been demonstrated unequivocally by insect-to-insect passage in the laboratory It is possible that multiplication in insects in nature is a common event, especially where a sucking insect is the natural vector for the virus It has not been technically possible to carry out quantitative studies

The Virus of CO_2 Sensitivity in Drosophila. The fourth variety of insect virus is a lone example (l'Heritier, 1958). This virus multiplies in injected flies and can be transmitted by a female fly to all offspring. The infection in itself is not lethal but renders the flies susceptible to CO_2 narcosis. Its mode of transmission from one generation to another is complex and comparable in mechanism with the transmission of prophage in bacteria.

So far, virus infections have not been found in other invertebrates except for the incrimination of the lungworm as the intermediate host for swine influenza (Shope, 1943), and this has been demonstrated only indirectly. Similarly, no known virus infections of Protozoans have been described The killing agent (kappa) found in paramoecia (Sonneborn, 1949) is structurally similar to rickettsiae

LATENT OR PERSISTENT VIRUS INFECTIONS

A virus which is so virulent that it invariably kills its host creates a condition of increasing difficulty for its own survival When a highly virulent myxoma virus was introduced into the wild rabbit populations of Australia (Fenner, 1957), the first waves of the ensuing epizootic were devastating However, the virulent strain tended to die with its victims, and less virulent derivatives began to appear, which were a major factor in subsequent waves. By a further extension of this adaptive process, variants may well appear which cause less and less inconvenience to the

host and, finally, some which cause none at all Such infections are called latent, and they are frequently persistent and form the main reservoir of viruses in nature. A true understanding of latency can be obtained only by studying infection at a cellular level, and this has been successful so far only with bacterial viruses.

Latency with Bacterial Viruses Bacteriophage latency is of two varieties, called (1) persistent infection and (2) lysogeny

PERSISTENT INFECTION A contaminating virus may persist in a continuously growing culture of bacteria over long periods of time if the bulk of the bacteria are resistant to virus infection and if susceptible mutants are thrown off occasionally (Benzer et al., 1950) The virus may persist at a low level in this culture by multiplying in these rare susceptible bacteria. This type of persistent infection is characterized by the fact that the virus can be removed readily and permanently by growing the bacteria in the presence of an antiviral serum The serum prevents the re-infection of new cells and shows that the propagation of mature virus is necessary for maintaining the infected state of the culture

LYSOGENY is a condition in which the ability of a bacterium to produce bacteriophage is hereditary The condition may be induced in a bacterium by infection with a temperate phage.

There are two principal types of bacteriophages: virulent and temperate (Lwoff, 1953) Virulent phage kills the bacterium, multiplies within it and finally lyses the cell Temperate bacteriophage may enter a host cell and pursue either of two courses In one case, the virus behaves like a virulent bacteriophage in all respects, in the other case, there is a series of events leading to the condition of lysogeny. The cell is not killed but survives and divides Some of the early progeny are lysogenic, and some are not Eventually, the lysogenic bacteria give rise to stable lysogenic clones in which each bacterium is lysogenic Lysogenic bacteria give rise to progeny which are normal in appearance and are in turn lysogenic However, a small proportion will give rise to viruses of the original infecting type This proportion may be small (10^{-6}) and depends on a number of factors, such as the kind of virus and the condition of the culture. Certain physical and chemical agents, called inducers (Lwoff, 1953), have the capacity to initiate phage production and lysis in all lysogenic cells, but a lysogenic bacterium cannot be lysed by either the original or closely related phages

The nonviral form of the temperate phage as carried in the bacterium is called prophage, and this may consist largely of the DNA of the infecting organism, which replicates without forming mature phage particles. In a bacterium which is about to become lysogenic, the infecting DNA does not replicate as usual but probably enters into some sort of close association with the bacterial chromosome, where it replicates at the same rate as purely bacterial regions of host DNA The region in which the prophage is located can be determined on the bacterial chromosome map (Lederberg and Lederberg, 1953), and there is evidence of homologies between bacterial and viral DNA in this region This region of the chromosome may be introduced into other bacteria by the process of recombination or by transduction The presence of prophage does not appear to harm the bacterial economy The use of inducers, such as ultraviolet light, may upset this harmonious accord, causing the prophage to go into a state of vegetative reproduction with the formation of mature particles However, prophage may mutate occasionally so that, on induction, no virus particles appear or they may be defective and lack the property of infectivity (Jacob and Wollman, 1956). Sometimes a prophage controls the production of certain bacterial antigens or other specific products such as toxin production by diphtheria (Groman, 1955) Even defective phages may control antigen production, in which case the role of the virus in the bacterial economy might pass unsuspected, detectable only because the bacterium is immune to re-infection with the same virus

Lysogeny is a state of parasitism at the genetic level There may be multiple lysogenic viruses in a single bacterium, and the main reservoir of bacterial viruses in nature consists of agents occurring in the lysogenic state.

Latent Infection of Animals Latent and persistent infections are common in animals The complex character of the host provides a multiplicity of ways in which the in-

fection may persist and, at the same time, makes it enormously difficult to discover the basis of the latency It is possible that some special virus form analogous to provirus may exist, or the viral agent may undergo loss of destructive properties, or the virus may be held in an extracellular or intracellular location where it is stable, or the rate of virus multiplication and cell destruction may be greatly slowed down so that cellular replacement can keep up with it Possibly all these mechanisms play a role in one or another latent infection, but it is difficult or impossible to understand the mechanism without single cell studies, the development of which is just beginning

In the following examples, a number of latent or modified infections are described briefly, which may give an idea of the breadth of problems involved in animals

Influenza The mechanism of persistence of influenza virus between human epidemics is unknown, and conceivably strains may be kept going in man by means of subclinical or sporadic infections The outbreaks of the very similar virus in swine occur in such a manner that Shope (1943) does not believe that the epizootic picture can be explained entirely by animal-to-animal transmission. Shope has suggested an additional cycle in which lungworm parasites of swine become infected, give off infected ova which are ingested by earthworms, which in turn are eaten by swine, after which the latently infected lungworms establish themselves in the lung The latent infection can be changed to an overt one by administering various kinds of shock treatment to the pig According to Shope, the influenza virus is latent in both the lungworm and the earthworm, and it cannot be demonstrated directly in either by the most delicate technic. Since the reversion from latency can occur only in the intact swine, the nature of the latent condition has not been clarified.

Herpes Simplex. The primary host for this virus is man Infection usually takes place early in life, sometimes with an extensive stomatitis This is usually, possibly always, followed by a latent infection which can persist throughout life, being activated by various physiologic states (such as menstruation) or affected by the occurrence of bacterial infections in the host. The recurrent form of the disease consists of vesicular lesions, frequently of the lip, and there is some suggestion that the recurrent vesicles always occur in the same area Very likely the dermal cells of the lip are latently infected There is no laboratory model of this infection, and the study of the herpes virus in single cells is just beginning.

Lymphocytic Choriomeningitis. This virus induces a chronic asymptomatic infection in mice The infection may spread in a mouse colony by the intra-uterine route There is doubtless some mortality from infection, even when it occurs early, but once the chronic condition is established superinfection is not fatal (Traub, 1939). These mice do not make neutralizing antibodies against the virus, suggesting that the early infection induced some form of immune tolerance.

Psittacosis. This infection, somewhat like LCM, occurs in nestling psittacine birds, and some of them die The survivors become healthy and, while they show no symptoms, often virus may be found in the spleen where it may be multiplying slowly. Physiologic and environmental effects are able to upset this balance, and crowding, for example, will cause some birds to become ill and excrete virus The chronic state does not confer any immunity (cf Chap 33)

Coxsackie and Echo Virus Infections In a search for agents in stools, it was found unexpectedly (Dalldorf and Sickles, 1948) that the intestine often contained a great many viruses which were pathogenic for the suckling mouse. Some of these viruses are the causative agents of specific disease syndromes, such as epidemic pleurodynia, but many of them are apparently harmless agents which multiply in the intestine without causing symptoms. There is little evidence that such infections are prolonged

A somewhat different situation is found with mouse poliomyelitis virus in mouse colonies where it is found that infection with one of several viruses of this group takes place early in life by contamination from the parents and, thereafter, the virus multiplies in the intestinal tract for long periods, without producing symptoms, except in that occasional mouse where the virus breaks through

in the central nervous system where it may cause a fatal infection (Olitsky, 1940)

Tumor Viruses. This class of agent furnishes the best examples of what may properly be termed true latency The infectious agent may come through the egg, through maternal milk or in other ways The virus grows in certain cells for long periods without giving any evidence of its presence There is some evidence (see later section on tumors) suggesting that there is a relationship of the virus with cellular genetic material like that which occurs with lysogeny Information about tumor viruses is too incomplete to furnish many interesting details at this time One example of latency often cited is that of Shope Papilloma virus in domestic rabbits, in which tumors can be incited by virus but where no detectable virus growth is found This may merely be a quantitative difference in the amount of virus produced (Beard 1956)

Other examples of latency could be cited, but they would add little to the picture given by the foregoing diseases In clinical medicine, latency is used in an even broader sense, including conditions which occur in the course of normal infection Almost all the virus diseases of childhood, for example, have a long silent (latent) period of incubation between the initial infection and the onset of the main manifestations of the disease There are at least two factors in this In almost all cases, the virus may first multiply in a location such as the pharynx where no symptoms (at least not classic symptoms) arise This may be followed by a spread through the blood stream of virus to other cells Here again as is often witnessed in laboratory examples, there is another delay between the peak of virus multiplication and the occurrence of cellular pathology The term "latency" is sometimes extended to cover examples of asymptomatic infection In influenza epidemics, for example, a large proportion of those infected have no symptoms, even though virus multiplies in their respiratory tract, and an antibody response ensues Such silent infections occur with many viruses, and the cause is obscure The infections are not necessarily persistent.

There is another large group of viruses which are latent in tissues and are found only by manipulation of these tissues, either by serial animal-to-animal passage or through cell culture It has been common experience for years that, when an organ was ground and passed serially, very often a pathogenic virus would appear which was frequently lethal Examples are virus III, in rabbit testis (Rivers and Tillett, 1924), and pneumonia virus of mice (Horsfall and Hahn, 1940). However, the extraordinary number of such viruses was not appreciated until cell culture was undertaken on a large scale

The culture of epithelial cells of monkey kidney, for example, has been carried on on a very large scale as a means of producing poliomyelitis vaccine Incidental to this and other work with monkey kidneys, a number of viruses have been found which appeared spontaneously in kidney cell culture (Hull et al , 1956) and brought about lysis of the cells Most of these agents, once they appeared, were readily propagated in series, but few have been linked definitely with any normally occurring infection The meaning of these findings is therefore not clear.

Somewhat similar results were obtained with test tube culture of the epithelium of surgically excised tonsils (Rowe et al , 1953) From such material, both fibroblasts and epithelium may grow and often, after even 6 or 8 weeks, the cells begin to die suddenly, and it can be shown that this is due to a virus which is readily propagated on HeLa or other cells Viruses are found in a large proportion of tonsils and they fall into a homogeneous group which has been subdivided on the basis of antigenic types into a number of subgroups (cf Chap 30) Some, such as type 3, are known to cause an epidemic type of respiratory infection, but others are associated with no recognized disease

No studies have been carried out as yet to determine the nature or the frequency of occurrence of this latency in the individual cells of a tonsil, but it appears to be a rare event.

The study of latency at a cellular level, in tissue culture, is just beginning, and it is too soon to draw conclusions from the studies which have already been carried out Ackermann and Kurtz (1955) showed that, if a culture of HeLa cells is heavily infected with poliomyelitis, most of the cells die, but a few survivors may grow out, and they may be maintained as infected cells in the presence of virus if sufficient virus antibody is included

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they can change host cell character must include the induction of unrestrained growth

The most convincing evidence in favor of the virus etiology of tumors is the growing list of neoplasms in which a viral agent is clearly implicated. This now includes classic types of tumors, such as mammary carcinoma and lymphatic leukemia (of mice) The status of the known examples of virus-induced tumor will be reviewed briefly

Virus-Induced Tumors of Birds. The domestic fowl is a bird with a very high incidence of spontaneous mesodermal tumors, many of which are virus-induced and include fibromas, sarcomas, myxomas, osteomas, lymphoid tumors and leukemias The causative agents are as varied as the tumors, each inducing growths of the sort from which it was procured The pioneer work in the field was done by Rous (1911) who demonstrated the tumor-inducing capacity of tumor filtrates from several different tumors This touched off a lively controversy as to the relevance of the virus to tumor growth and, in subsequent work, much attention was given to one of these agents, called Rous sarcoma virus, or RSV

Rous sarcoma virus is about 70 to 80 mμ in diameter and can be seen in the electron microscope but is fragile and never has been purified to any extent or analyzed chemically The cells from tumors all yield virus (Rubin, 1955) but at a slow rate, the average being something like one virus particle per 100 cells per hour. A most interesting point is its susceptibility to x-ray and ultraviolet irradiation, in which it differs radically from other animal viruses It has a sensitivity like other viruses to x-ray but *extraordinary resistance* to UV (Rubin, personal communication) This pattern of reaction is like that of the temperate viruses, and this behavior in respect to resistance to UV is interpreted with phage as being due to homologies between host and virus genetic substances, permitting rapid replacement of injured virus nucleic acid This type of radiosensitivity, the very narrow host range of the virus and the fact that RSV-infected fibroblasts divide is the best evidence which exists so far for some sort of provirus in the animal virus world

Rous sarcoma virus produces tumors with difficulty, if at all, in such related species as ducks, pheasants, etc It readily infects the chorio-allantoic membrane where it is capable of inducing both mesothelial and endothelial tumors (Rubin, 1955) Recently, it has become possible to infect chick fibroblasts in monolayers with production of plaques of tumor cells (Manaker and Groupé, 1956, Rubin, unpublished experiments)

Our knowledge of the serology of the virus is not very satisfactory. It can be neutralized by specific antiserum in the absence of complement But practically all barnyard fowl have antibodies to the virus, and the level increases with age Since this is the only host for the virus, neutralization tests have been complicated If a bird bears a virus-type sarcoma, the circulating antibody level is reduced, but, if the tumor is slow-growing or regressing, the blood level may be high The serology of the virus as tested on the chorio-allantoic membrane is straightforward

The vast majority of spontaneous fowl tumors cannot be passed directly by means of filtrates However, if the spontaneous tumor is first passed from bird to bird by means of whole cells, many become altered, so that filtrates are infectious In carcinogen-induced tumors of fowl this ability to be passed by filtrates does not occur even with prolonged transfer

There is no doubt about a causal relationship between virus and tumor in the case of the Rous sarcoma virus The response to the virus is rapid, a specific type of tumor is produced, and tumor induction can be obtained in vitro There are many other tumors of chickens, such as malignant lymphoma and leukemia, which involve the blood-forming organs, and these too are caused by viruses Some of these viruses, such as the one which causes malignant lymphoma, are very widespread in the normal fowl population, and there is evidence that the virus may spread through the egg

Kidney Tumor of the Leopard Frog. The leopard frog, which has a wide distribution throughout the continent of North America, frequently develops a spontaneous carcinoma of the kidney The tumor is quite malignant (Lucké, 1938) and may be maintained in frogs by grafting Filtrates of tumor will produce the disease, and the tumors contain small particles which, in section, appear to

in the medium to reduce greatly the probability of cell-to-cell transmission of infection (Vogt and Dulbecco, 1958). A similar situation can be demonstrated with NDV and HeLa cells (Ciecura et al, 1957; Puck, personal communication), in which case a large portion of the growing cells were shown to carry virus even in the absence of antibody The cells were not the wild type and were more difficult to infect than the normal ones. This infection, too, could be cured by antibody

These experiments with isolated cells so far emphasize the possible importance of circulating antibody, of persistent, slowly progressing infection, and selection of partially resistant host cells as possible factors in persistent virus infection

Latency in Plant Virus Infections Latency of infection in plants is characterized by the presence of virus unaccompanied by or with reduced symptoms. The classic example is the King Edward potato which is propagated vegetatively, is always full of virus but appears to be normal. When the potato is raised from seed, it does not contain the virus.

Some plants wither after infection but, before dying, send out new shoots which appear to be healthy These new tissues contain a reduced amount of virus and cannot be superinfected, but the reason for this immunity is not clear.

Virus Latency in Insects. Latency in insects is poorly understood because of poor titration technics for insect viruses It is quite common for epizootics to break out and decimate old laboratory-grown insect colonies, even after years of apparent health Apparently, some chemicals can act as inducers (Bergold, 1953)

VIRUSES AND TUMOR FORMATION

The importance of viruses in the etiology of malignant tumors has only gradually come to be widely recognized The increased importance of the virus theory stems both from research on tumors as well as from the development of virology. There is now an impressive group of tumors in which a virus plays an obvious role Oncologists are more interested than formerly in genetic changes, such as mutation, as a factor in cancer etiology, and the bacterial virologists have furnished clear-cut examples of the ways in which viruses can induce heritable changes in cells

The suggestion that tumors might have an infectious etiology was given substance by Rous (1911) with the discovery that filtrates of chicken tumors had the capacity to induce tumor formation in injected fowl The idea of a viral etiology for tumors did not go unchallenged It was said that chicken tumors were atypical tumors, that the viruses were passengers rather than inciters of the disease and, when similar agents were not found shortly in mammalian cancers, interest in virus etiology receded

Attention was turned to cancer induced by tars and other chemicals. After the isolation and the synthesis of carcinogenic compounds from coal tar was accomplished, a great number of other pure substances were found which were carcinogenic, yet the mechanism by which cancers were induced remained obscure A number of strongly carcinogenic chemicals were found to be potent mutagens as well Ultraviolet light and x-rays also have this dual capacity, and it has long been known that cancer cells show marked disorder of the normal chromosome structure. These facts and the obvious heritable character of the cancerous quality in cells have contributed to a revival of interest in the theory that cancer is due to something like somatic mutation This view does not exclude the possibility that *either* chemical agents or viruses could act as the mutagenic agent under different circumstances

Virus studies in other fields have provided sufficient evidence about the behavior of viruses, especially bacterial viruses, to make the case for virus etiology of tumors a plausible one As recounted under the section on latent virus infections and in the chapter on bacteriophages, there are a number of examples of lysogeny in which the virus persists in the cell in nonvirus form through an indefinite number of cell replications without ever being detected in the active form Such a prophage may cause the phenotype of the host cell to change (production of new antigens or new toxins) in quite definite heritable ways Nothing inherently different is required of tumorigenic viruses except that the ways in which

tive to ether. It is antigenic in rabbits, producing sera of weak or dubious neutralizing value It appears to be nonantigenic in mice It never has been purified or characterized chemically to any satisfactory degree It appears to multiply on passage in eggs, but the virus does not lend itself to accurate titration

The agent in milk (or from other organs) can cause breast cancer in mice by feeding, if fed in the early part of life, but the specific effect does not occur for many months (8 to 20, depending on the mouse strain) During this latent period there is no evidence of infection, and efforts to shorten the latent period have been unsuccessful In mice that are susceptible to the agent, the virus appears not only in the breast and the breast tumor but also in many other organs as well as in the blood However, it does not infect in utero The virus may reproduce in and be carried by strains of mice in which the incidence of cancer is low

At least two other factors are essential for the virus to be able to express itself in inducing cancer One is a hormonal effect (Huseby et al, 1946) Mammary carcinoma has a low incidence in the male If a male is castrated and then given estrogenic hormone, the susceptibility to virus-induced cancer becomes much greater If the male mice are of a high cancer line, the presence of virus plus hormonal treatment will cause an incidence of tumors equivalent to that in the virus-infected female In the absence of the virus, the tumors do not appear. Also, males of the low line show no tumors unless the virus is present

A second auxiliary effect is the genetic character of the host The virus, given to susceptible mice, produces a high incidence In low cancer (resistant) mice (Dmochowski, 1948), the incidence remains low In hybrids, the results are sometimes intermediate The resistance is further shown by the fact that virus given even in adulthood to susceptible mice will increase the incidence of cancer, while it has no effect in resistant adults It is clear, too, that the hormonal effect may be controlled genetically

The agent has been found naturally in a number of high-incidence inbred lines It has not been found in low-tumor lines (Dmochowski, 1951) The removal of the virus from a population by fostering lowers the cancer incidence markedly, but there remains a core of unaffected tumors The nature of this residue is unclear Histologically, the tumors in the low-cancer line are not very different from those occurring as a result of the milk factor. It has not been possible to demonstrate a maternal factor in the transmission of these low-incidence tumors, and no filterable agent has been obtained from them which incites tumor formation However, it cannot be ruled out that they are virus-induced, and perhaps only the proper means of demonstrating the agent is lacking, or the "provirus" may be defective and unable to produce a mature form

In summary, a high proportion of the mammary carcinomas which occur in high-tumor mouse lines is due, in part at least, to a virus The physical characteristics of the agent are as yet poorly defined The agent normally enters the newborn mouse with maternal milk and causes an infection which may involve a great many organs, but this infection has no detectable manifestation until fairly late in the life of the animal A certain genetic constitution, the details of which are not clear, and certain hormonal conditions, as well as the presence of the virus, are necessary for the tumor to appear These peculiarities of virus behavior the early symptomless infection, the long latent period and the need for specific genetic and physiologic conditions, and the production of a classic neoplasm have gone a long way in dispelling the prior objections to the virus etiology of tumors

Mouse Leukemia In addition to a wide array of tumors, mice also suffer from a number of blood dyscrasias, particularly leukemias The leukemias are now widely regarded as another type of malignant tumor. Lymphatic leukemia has been especially well studied in mice, and strains of mice have been developed through selection and inbreeding in which the incidence of spontaneous leukemia is as high as 95 per cent One such strain is called the A-K line

Mouse leukemias have frequently been studied by grafting cells from leukemic to nonleukemic animals and, when this has been done successfully, the disease develops in the recipient within a relatively short time In very young mice, an exceedingly acute and rapidly fatal disease is produced The trans-

be very much like viruses in structure (by electron microscopy)

Papillomatosis of Rabbits. For many years, the only clear-cut examples of virus-induced tumors were those of domestic fowl until Shope (1933) described the isolation of a virus from naturally occurring papillomas of wild North American rabbits The papillomas are nonmalignant, warty-type growths which appear on the ears and the backs of rabbits, and sometimes these excrescences are considerably cornified If the tumors are ground, filtered and titrated by inoculation into the skin of either wild or domestic rabbits, tumors are induced In the wild rabbit, these are full of virus, while in the domestic rabbit, little or no virus is found If the cells of these tumors are stained with fluorescent antiviral antibody, the virus is seen to exist mainly in the *nuclei* of the cells of the keratohyaline layer of the dermis (Noyes and Mellors, 1957) In domestic rabbits, the amount of virus found by this method is small When domestic rabbit tumors are transferred to other domestic rabbits by grafting, the host rabbits develop antiviral antibodies, showing the existence of virus antigens in the tumor Shope has described the virus in the domestic rabbit as being in a masked condition, although virus may simply be there in very small quantity (Beard, 1956)

Virus-induced papillomas are usually benign growths, but, after long periods of time (Rous and Beard, 1935, Kidd and Rous, 1940), they may become highly malignant and metastasize At this stage, virus cannot be detected in the tumor directly, but if the tumor is grafted onto a new host by means of whole cells, the new bearer responds by developing antibodies against the virus, showing continued presence of the antigens in the tumor That the virus (or antigen) is not necessary for continued malignancy is shown by the fact that, after prolonged transfer from rabbit to rabbit by whole cells, all serologic evidence of a virus being present may be lost (Rous et al , 1952), while the malignant characteristics of the tumor remain unchanged

Still another type of virus-induced tumor of rabbits is the fibroma (Shope, 1932) After inoculation of this agent, fairly typical and rapidly growing fibrous tumors are formed However, these are purely benign tumors, and they frequently regress and disappear spontaneously. Sometimes the virus from these regressing tumors also shows changes and will subsequently induce only a mild inflammatory disease in other rabbits. Because of these vagaries, this tumor will not be considered further. It may also be noted that this virus is related serologically to the agent causing myxomatosis in rabbits Berry and Dedrick (1936) found that heat-killed myxoma virus has the capacity, when injected with live fibroma virus, of permanently affecting the genotype of the latter, and it modifies the type of tumor produced in the direction of the tumor from which the heated extract was obtained

Up to this point it was frequently said that the virus-induced tumors were a different class of neoplasm than those which are not virus-induced The discovery of a viral agent from mouse mammary cancer was notable because this was a classic neoplasm Mammary cancer had been long studied from the genetic standpoint to determine the mode of its inheritance Little and his associates (Murray and Little, 1936) at Bar Harbor selectively inbred mice and obtained lines of animals which had a high and a low incidence of spontaneous mammary cancer They observed that, when these high and low cancer strains were crossed, the incidence of tumors in the offspring was greater if the mother, rather than the father, was from the high tumor line This maternal effect could be explained in three ways: it was a transfer of cytoplasmic factors by the ovum, an effect in utero, or something transferred in the milk Bittner (1936) found evidence for the early transfer of virus through the milk It was found that, if the offspring from a high cancer mother were removed immediately after birth and foster-nursed by a low cancer mother, the incidence of cancer in the offspring would be low, of the same order as the low line However, the contrary experiment of weaning offspring of low-line mothers to high-line foster mothers did not produce a dramatic rise in the expected incidence of breast cancer

Bittner showed that it was an agent in milk which had this effect The agent (Dmochowski, 1953) as it exists in milk is of unknown size but is sensitive to heat (inactivated at 60° C. for 30 minutes), and it is insensi-

tive to ether. It is antigenic in rabbits, producing sera of weak or dubious neutralizing value. It appears to be nonantigenic in mice It never has been purified or characterized chemically to any satisfactory degree It appears to multiply on passage in eggs, but the virus does not lend itself to accurate titration

The agent in milk (or from other organs) can cause breast cancer in mice by feeding, if fed in the early part of life, but the specific effect does not occur for many months (8 to 20, depending on the mouse strain) During this latent period there is no evidence of infection, and efforts to shorten the latent period have been unsuccessful In mice that are susceptible to the agent, the virus appears not only in the breast and the breast tumor but also in many other organs as well as in the blood However, it does not infect in utero The virus may reproduce in and be carried by strains of mice in which the incidence of cancer is low

At least two other factors are essential for the virus to be able to express itself in inducing cancer One is a hormonal effect (Huseby et al, 1946) Mammary carcinoma has a low incidence in the male If a male is castrated and then given estrogenic hormone, the susceptibility to virus-induced cancer becomes much greater If the male mice are of a high cancer line, the presence of virus plus hormonal treatment will cause an incidence of tumors equivalent to that in the virus-infected female In the absence of the virus, the tumors do not appear Also, males of the low line show no tumors unless the virus is present

A second auxiliary effect is the genetic character of the host The virus, given to susceptible mice, produces a high incidence In low cancer (resistant) mice (Dmochowski, 1948), the incidence remains low In hybrids, the results are sometimes intermediate The resistance is further shown by the fact that virus given even in adulthood to susceptible mice will increase the incidence of cancer, while it has no effect in resistant adults It is clear, too, that the hormonal effect may be controlled genetically

The agent has been found naturally in a number of high-incidence inbred lines It has not been found in low-tumor lines (Dmochowski, 1951) The removal of the virus from a population by fostering lowers the cancer incidence markedly, but there remains a core of unaffected tumors The nature of this residue is unclear Histologically, the tumors in the low-cancer line are not very different from those occurring as a result of the milk factor It has not been possible to demonstrate a maternal factor in the transmission of these low-incidence tumors, and no filterable agent has been obtained from them which incites tumor formation However, it cannot be ruled out that they are virus-induced, and perhaps only the proper means of demonstrating the agent is lacking, or the "provirus" may be defective and unable to produce a mature form

In summary, a high proportion of the mammary carcinomas which occur in high-tumor mouse lines is due, in part at least, to a virus The physical characteristics of the agent are as yet poorly defined The agent normally enters the newborn mouse with maternal milk and causes an infection which may involve a great many organs, but this infection has no detectable manifestation until fairly late in the life of the animal A certain genetic constitution, the details of which are not clear, and certain hormonal conditions, as well as the presence of the virus, are necessary for the tumor to appear These peculiarities of virus behavior, the early symptomless infection, the long latent period and the need for specific genetic and physiologic conditions and the production of a classic neoplasm have gone a long way in dispelling the prior objections to the virus etiology of tumors

Mouse Leukemia. In addition to a wide array of tumors, mice also suffer from a number of blood dyscrasias, particularly leukemias The leukemias are now widely regarded as another type of malignant tumor Lymphatic leukemia has been especially well studied in mice, and strains of mice have been developed through selection and inbreeding in which the incidence of spontaneous leukemia is as high as 95 per cent One such strain is called the A-K line

Mouse leukemias have frequently been studied by grafting cells from leukemic to nonleukemic animals and, when this has been done successfully, the disease develops in the recipient within a relatively short time In very young mice, an exceedingly acute and rapidly fatal disease is produced The trans-

mission of leukemias in this way is similar to the propagation of solid tumors by grafting. Immune tolerance factors in the recipient play a role The leukemias transfer readily only to closely allied strains or better to an inbred line of the donor variety.

In recent years (Gross, 1956), a way has been found to transfer the disease from one animal to another by means of filtrates of adult leukemic tissue. It is necessary to inoculate the filtrates into newborn mice, not more than 12 hours old, if the virus is to have any effect. The leukemia then appears in mice (even in strains with a normally low incidence of the spontaneous disease) but it appears only after many months The type of disease is that found in the donor mouse

There are several obvious similarities to the behavior of the milk factor. The virus must be given early to be effective It does not produce any overt disease for many months The effect of genetic constitution of the mouse on the outcome is also clear, inasmuch as some strains of mice give a much higher number of takes than others. Relatively little is known about the physical characteristics of the virus

In recent years, several other tumors of viral etiology have been found in the mouse When cell-free extracts of mouse leukemia cells are prepared (Gross, 1955, Stewart et al, 1957) and inoculated into very young mice, other tumors sometimes appear, such as primary tumors of the parotid, the submaxillary gland and other organs, indicating the presence of other tumor-producing agents Friend (1957) recently described a virus which produces leukemia in adult mice

For the few tumors of proved virus etiology, there are great numbers of tumors in which very extensive efforts to prove such an etiology have failed The reason for the failures may be that the tumors do not contain viruses, and viruses have nothing to do with their origin, or it is possible that a virus-inciting agent is of such subtle nature that ways have not been found to demonstrate it. Some of the difficulties of virus demonstration may be seen in the foregoing examples. It has been pointed out repeatedly that the tumors obtained with papilloma virus in domestic rabbits would pass as nonviral in origin if the domestic rabbit were the only experimental host.

There is no longer much question about the cause and effect relationship between some viruses and certain tumors. The causative agent has all the attributes of a virus. Its effect on the cell in the case of the Rous sarcoma virus is direct, rapid and specific. There is no other postulated agent which has these properties As was noted in the beginning of this section, there is also evidence from other sources to indicate that a virus can have a profound effect on the functional properties of the cell, especially when it is the type of virus which does not destroy the cell We can think of tumorigenic viruses as being analogous to the temperate rather than the virulent bacteriophages The action of initiating cancerous growth could be due to a disturbance of the normal cellular genetic apparatus Once initiated, it is not necessary to postulate continued presence of the virus to maintain the condition, although it may be there in provirus form. It is not necessary to assume that only a virus could have this same disturbing action on the cell Thus, in accepting the virus theory of the origin of cancer, it does not appear to be necessary to accept it as an exclusive theory. The investigation of tumor viruses is really just beginning and, with the advent of new tissue culture technics, it is not a wild speculation to guess that discoveries in this field will be of even greater interest than those with the viruses which cause acute necrosis and cell death

REFERENCES

Ackermann, W W, and Francis, T, Jr, 1954, Characteristics of viral development in isolated animal tissues in Advances in Virus Research, vol 2, pp 81-108, New York, Acad Press

Ackermann, W W, and Kurtz, H, 1955, Observations concerning a persisting infection of HeLa cells with poliomyelitis virus, J Exper Med 102, 555-565

Ada, G L, 1957, Ribonucleic acid in influenza virus in The Nature of Viruses, pp 104-122, Ciba Foundation Symposium, London, Churchill

Ada, G L, and Gottschalk, A, 1956, The component sugars of the influenza-virus particle, Biochem J 62, 686-689

Ada, G L, and Stone, J D, 1950, Electrophoretic studies of virus-red cell interaction Mobility gradient of cells treated with viruses of the influenza group and the receptor-destroying enzyme of V. cholerae, Brit J Exper Path 31, 263-274

Adams, M H, and Park, B H, 1956, An enzyme produced by a phage-host cell system II The

properties of the polysaccharide depolymerase Virology 2, 719-736
Anderson, S G, 1948, Mucins and mucoids in relation to influenza virus action I Inactivation by RDE and by viruses of the influenza group, of the serum inhibitor of haemagglutination Australian J Exper Biol & M Sc 26, 347-354
Anderson, T F, and Doermann, A H, 1952, Sonic reactivation of antiserum-neutralized bacteriophage T_3, J Bact 63, 291-292
Andrewes, C H, 1954, Nomenclature of viruses Nature, London 173, 620 621
Andrewes, C H, Bang, F B, and Burnet, F M, 1955, A short description of the myxovirus group (influenza and related viruses), Virology 1, 176-184
Andrewes, C H, and Elford, W J 1933, Observations on anti-phage sera I "The percentage law" Brit J Exper Path 14, 367-376
Andrewes, C H, and Horstmann D M 1949, The susceptibility of viruses to ethyl ether, J Gen Microbiol 3, 290-297
Bachtold, J G, Bubel, H. C, and Gebhardt L P 1957, The primary interaction of poliomyelitis virus with host cells of tissue culture origin, Virology 4, 582-589
Bader, E, Jr, and Commoner B 1956 The effect of tobacco mosaic virus biosynthesis on the nucleic acid content of tobacco leaf, Virology 2, 13-28
Beard, J W, 1956, The fallacy of the concept of virus "masking" A review Cancer Res 16, 279-291
Beard, J W, Sharp, D G, and Eckert E A, 1955 Tumor viruses in Advances in Virus Research, vol 3, 149-197, New York Acad Press
Bedson, S P, and Bland, J O W, 1932, A morphological study of psittacosis virus with the description of a developmental cycle, Brit J Exper Path 13, 461-466
Benzer, S, Delbruck, M Dulbecco R, Hudson, W, Stent, G S, Watson, J D, Weidel W, Weigle, J J, and Wollman, E L, 1950 A syllabus on procedures, facts, and interpretations in phage, in Viruses, Delbruck, M (ed), pp 100-147, Pasadena, California Institute of Technology
Bergold, G H, 1953, Insect viruses in Advances in Virus Research, vol 1, pp 91-139, New York, Acad Press
Berry, G P, and Dedrick, H M 1936, A method for changing the virus of rabbit fibroma (Shope) into that of infectious myxomatosis (Sanarelli), J Bact 31, 50 51
Beveridge, W I B, and Burnet, F M, 1946, The cultivation of viruses and rickettsiae in the chick embryo Med Res Council, Special Report series No 256, pp 1-92, London, His Majesty's Stat Off
Bittner, J J, 1936, Some possible effects of nursing on the mammary gland tumor incidence in mice, Science 84, 162
Black, L M, 1953, Viruses that reproduce in plants and insects, Ann New York Acad Sc 56, 398-413
Bloch, D P, Morgan, C Godman, G C, Howe, C, and Rose, H M, 1957, A correlated histochemical and electron microscopic study of the intranuclear crystalline aggregates of adenovirus (RI APC virus) in HeLa cells, J Biophys & Biochem [illegible] 3, 1-8
Breitenfeld, P M, and Schäfer, W, 1957, Th[illegible] mation of fowl plague virus antigens in i[illegible] cells, as studied with fluorescent antibodies, [illegible]ogy 4, 328-345
Burnet, F M, 1942, The affinity of Newcast[illegible] ease virus to the influenza virus group, Aus[illegible] J Exper Biol & M Sc 20, 81-88
———, 1955, Principles of Animal Virology [illegible] York, Acad Press 486 pp
———, 1956, Structure of influenza virus, [illegible] 123, 1101-1104
Burnet, F M, and Anderson, S G, 1947, Th[illegible] antigen of guinea-pig and human red cells [illegible]tralian J Exper Biol & M Sc 25, 213-21[illegible]
Burnet, F M, and Boake, W C, 1946, The re[illegible] ship between the virus of infectious ectrom[illegible] mice and vaccinia virus, J Immunol 53, [illegible]
Burnet, F M, and Bull D R, 1943, Chan[illegible] influenza virus associated with adaptation t[illegible] ace in chick embryos, Australian J Exper [illegible] M Sc 21, 55-69
Burnet, F M, and Lind, P E, 1950, Haemoly[illegible] Newcastle disease virus II General charac[illegible] hemolytic action Australian J Exper Biol [illegible] Sc 28, 129-150
———, 1951, A genetic approach to variat[illegible] influenza viruses 4 Recombination of cha[illegible] between the influenza virus A strain NW[illegible] strains of different serological subtypes J [illegible] Microbiol 5, 67 82
———, 1954, Reactivation of heat inactivated [illegible]enza virus by recombination, Australian J [illegible] Biol & M Sc 32, 133-143
Burnet, F M, and Stone, J D, 1947, The rec[illegible] destroying enzyme of V(ibrio) cholerae, Aus[illegible] J Exper Biol & M Sc 25, 227-233
Casals, J, 1957, Viruses The versatile paras[illegible] The arthropod-borne group of animal virus[illegible] New York Acad Sc, Ser 2, 19, 219-235
Casals, J, and Brown, L V, 1954, Hemaggl[illegible] tion with arthropod-borne viruses, J Exper [illegible] 99, 429-449
Chu, L W, and Morgan, H R, 1950, Stu[illegible] the hemolysis of red blood cells by mumps [illegible] I The development of mumps virus hemolys[illegible] its inactivation by certain physical and ch[illegible] agents, J Exper Med 91, 393-402
Ciecura, S J, Marcus, P I, and Puck, T T, [illegible] The use of x-irradiated HeLa cell giants to [illegible] latent virus in mammalian cells, Virology [illegible] 428
Cohen, S S, 1947, The synthesis of bacterial [illegible] in infected cells, Cold Spring Harbor Symp [illegible] Biol 12, 35-49
———, 1949, Growth requirements of bacter[illegible] ruses, Bact Rev 13, 1-24
———, 1953, Studies on controlling mechani[illegible] the metabolism of virus-infected bacteria, [illegible] Spring Harbor Symp Quant Biol 18, 221-
———, 1957, The biosynthesis of nucleic ac[illegible] some microbial systems in The Chemical B[illegible]

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REFERENCES

Ackermann, W W, and Francis, T, Jr, 1954, Characteristics of viral development in isolated animal tissues *in* Advances in Virus Research, vol 2, pp 81-108, New York, Acad Press

Ackermann, W W, and Kurtz, H, 1955, Observations concerning a persisting infection of HeLa cells with poliomyelitis virus, J Exper Med *103*, 555-565

Ada, G L, 1957, Ribonucleic acid in influenza virus *in* The Nature of Viruses, pp 104-122, Ciba Foundation Symposium, London, Churchill

Ada, G L, and Gottschalk, A, 1956, The component sugars of the influenza-virus particle, Biochem J *62*, 686-689

Ada, G L, and Stone, J D, 1950, Electrophoretic studies of virus-red cell interaction Mobility gradient of cells treated with viruses of the influenza group and the receptor-destroying enzyme of V *cholerae*, Brit J Exper Path *31*, 263-274

Adams, M. H., and Park, [illegible] H, 1956, An enzyme produced by a phage-host cell system II The

Corynebacterium diphtheriae to toxin production, J Bact *60*, 9-15

Gross, L., 1953, Induction of parotid carcinomas and/or subcutaneous sarcomas in C_3H mice with normal C_3H organ extracts, Proc Soc Exper Biol [redacted] Med *83*, 362-368

———, 1956, Viral (egg-borne) etiology of mouse leukemia Filtered extracts from leukemic C_{58} mice, causing leukemia (or parotid tumors) after inoculation into newborn C_{57} brown or C_3H mice Cancer *9*, 778-791

Habel, K, Hornibrook, J W, Gregg, N C, Silverberg, [redacted] J, and Takemoto, K K, 1955, The effect of anticellular sera on virus multiplication in tissue culture, Virology *3*, 7-29

Hallauer, C, 1946, Über den Virusnachweis mit dem Hirst-Test, Schweiz Ztschr f Path u Bakt *9*, 553-554

Hamers-Casterman, C, and Jeener, R 1957, An initial ribonuclease—sensitive phage in the multiplication of tobacco mosaic virus, Virology *3*, 197-206

Hanig, M, 1948, Electrokinetic change in human erythrocytes during adsorption and elution of PR8 influenza virus, Proc Soc Exper Biol & Med *68*, 385-392

Henle, G, Girardi, A., and Henle, W, 1955, A non-transmissible cytopathogenic effect of influenza virus in tissue culture accompanied by formation of noninfectious hemagglutinins, J Exper Med *101*, 25-41

Henle, W, Liu, O C, Paucker, K, and Lief, F S, 1956, Studies on host-virus interactions in the chick embryo-influenza virus system XII The relation between tissue-bound and liberated virus materials under various conditions of infection J Exper Med *103*, 799-822

d Herelle, F, 1917, Sur un microbe invisible antagoniste des bacilles dysentériques, Compt rend Acad sc *65*, 373-375

l'Heritier, Ph, 1958, The hereditary virus of drosophila *in* Advances in Virus Research, vol 5, pp 195-245, New York, Acad Press

Hershey, A D, and Chase, M, 1951, Genetic recombination and heterozygosis in bacteriophage, Cold Spring Harbor Symp Quant Biol *16*, 471-479

———, 1952, Independent functions of viral protein and nucleic acid in growth of bacteriophage, J Gen Physiol *36*, 39-56

Hershey, A D, Kalmanson, G, and Bronfenbrenner, J, 1943, Quantitative relationships in the phage-antiphage reaction Unity and homogeneity of the reactants, J Immunol *46*, 281 299

Hershey, A D, and Rotman, R, 1949, Genetic recombination between host range and plaque-type mutants of bacteriophage in single bacterial cells, Genetics *34*, 44-71

Hilleman, M R, Haig, D A, and Helmold, R J, 1951, The indirect complement fixation, hemagglutination and conglutinating complement absorption tests for viruses of the psittacosis-lymphogranuloma venereum group, J Immunol *66*, 115-130

Hirst, G K, 1941, The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus, Science *94*, 22-23

———, 1950, Receptor destruction by viruses of the mumps-NDV-influenza group, J Exper Med *91*, 161-175

Horsfall, F L Jr, 1954, On the reproduction of influenza virus Quantitative studies with procedures which enumerate infective and hemagglutinating virus particles J Exper Med *100*, 135-161

———, 1955, Reproduction of influenza viruses Quantitative investigations with particle enumeration procedures on the dynamics of influenza A and B virus reproduction J Exper Med *102*, 441-473

Horsfall, F L, Jr, and Hahn, [redacted] G, 1940, A latent virus in normal mice capable of producing pneumonia in its natural host, J Exper Med *71*, 391-408

Howe, C, Rose, H M, and Schneider, L, 1957, Enzymic action of influenza virus on human erythrocyte stroma components Proc Soc Exper Biol & Med *96*, 89-94

Hull, R N, Minner, J R, and Smith, J W, 1956, New viral agents recovered from tissue cultures of monkey kidney cells, Am J Hyg *63*, 204-215

Huseby R A, Smith, F W, and Bittner, J J, 1946, The genetic control of ovarian secretion as related to the incidence of mammary cancer in mice, Cancer Res *6*, 494

Isaacs, A, 1957, Particle counts and infectivity titrations for animal viruses in Advances in Virus Research, vol 4, pp 111-158, New York, Acad Press

Iwanowski, D, 1892, Über die Mosaikkrankheit der Tabakspflanze Bull de l'Acad Imp des Sci de St Petersbourg, *3* n s, 67-70

Jacob, F, and Wollman, E L, 1956, Recherches sur les bacteries lysogenes defectives I Determinisme genétique de la morphogenèse chez un bacteriophage tempéré Ann Inst Pasteur *90*, 282-302

Jeener, R, 1956, Ribonucleic acids and virus multiplication, Advances Enzymol *17*, 477-498

Jensen, K E, 1957, The nature of serological relationships among influenza viruses in Advances in Virus Research, vol 4, pp 279-310, New York, Acad Press

Jerne, N K, and Avegno, P, 1956, The development of the phage-inactivating properties of serum during the course of specific immunization of an animal Reversible and irreversible inactivation J Immunol *76*, 200-208

Kalmanson, [redacted] M, and Bronfenbrenner, J, 1943, Restoration of activity of neutralized biologic agents by removal of the antibody with papain, J Immunol *47*, 387-407

Kaplan, A S, 1955, The susceptibility of monkey kidney cells to poliovirus in vivo and in vitro, Virology *1*, 377-392

Kellenberger, E, and Sechaud, S, 1957, Electron microscopical studies of phage multiplication II Production of phage-related structures during multiplication of phages T_2 and T_4 Virology *3*, 256-274

Heredity, McElroy, W D, and Glass, B (eds), pp 651-685, Baltimore, Johns Hopkins Press

Colter, J S, Bird, H H, Moyer, A W, and Brown, R A, 1957, Infectivity of ribonucleic acid isolated from virus-infected tissues, Virology 4, 522-532

Commoner, B, and Basler, E, Jr, 1956, Variations in the nucleic acid composition of tobacco mosaic virus, Virology 2, 477-495

Curnen, E C, and Horsfall, F L, Jr, 1947, Properties of pneumonia virus of mice (PVM) in relation to its state, J Exper Med 85, 39-53

Curnen, E C, Pickels, E G, and Horsfall, F L, Jr, 1947, Centrifugation studies on pneumonia virus of mice (PVM) The relative sizes of free and combined virus J Exper Med 85, 23-38

Dalldorf, G, and Sickles, G M, 1948, An unidentified filterable agent isolated from the feces of children with paralysis, Science 108, 61-62

Dawson, I M, and Elford, W J, 1949, The investigation of influenza and related viruses in the electron microscope, by a new technique, J Gen Microbiol 3, 298-311

DeMars, R I, Luria, S E, Fisher, H, and Levinthal, C, 1953, The production of incomplete bacteriophage particles by the action of proflavine, and the properties of the incomplete particles, Ann Inst Pasteur 84, 113 128

Dmochowski, L, 1948, Mammary tumour inducing factor and genetic constitution, Brit J Cancer 2, 94-102

———, 1951, Spontaneous tumours in some low-cancer strain mice and the mammary tumour agent, Acta Unio internat contra cancrum 7, 230-233

———, 1953, The milk agent in the origin of mammary tumours in mice *in* Advances in Cancer Research, vol I, pp 103-172, New York, Acad Press

Doermann, A H, 1952, The intracellular growth of bacteriophages I Liberation of intracellular bacteriophage T_4 by premature lysis with another phage or with cyanide J Gen Physiol 35, 645-656

———, 1953, The vegetative state in the life cycle of bacteriophage Evidence for its occurrence and for its genetic characterization Cold Spring Harbor Symp Quant Biol 18, 3-11

Dulbecco, R, 1952, Production of plaques in monolayer tissue cultures by single particles of an animal virus, Proc Nat Acad Sc 38, 747-752

Dulbecco, R, Vogt, M, and Strickland, A G R, 1956, A study of the basic aspects of neutralization of two animal viruses, Western equine encephalitis virus and poliomyelitis virus, Virology 2, 162-205

Eagle, H, and Habel, K, 1956, The nutritional requirements for the propagation of poliomyelitis virus by the HeLa cell, J Exper Med 104, 271-287.

Eaton, M D, 1952, Observations on growth of virus and the energy-yielding activities of the host cell, Arch ges Virusforsch 5, 53-72

Eddy, B E, Rowe, W P, Hartley, J. W, Stewart, S E, and Huebner, R. J, 1958, Hemagglutination with the S. E Polyoma virus, Virology (in press)

Fenner, F, 1957, Myxomatosis in European rabbits—Evolutionary changes in an infectious disease Australian J Sc 19, (No 4a), 117-122

———, 1958, The biological characters of several strains of vaccinia, cowpox and rabbitpox viruses, Virology 5, 502-529

Fenner, F, and Burnet, F M, 1957, A short description of the poxvirus group (vaccinia and related viruses), Virology 4, 305-314.

Fraenkel-Conrat, H, 1956, The role of nucleic acid in the reconstitution of active tobacco mosaic virus, J Am Chem Soc 78, 882-883

———, 1957, Structure and infectivity of tobacco mosaic virus Harvey Lecture Series To be published

Fraenkel-Conrat, H, and Williams, R C, 1955, Reconstitution of active tobacco mosaic virus from its inactive protein and nucleic acid components, Proc Nat Acad Sc 41, 690-698

Francis, T, 1947, Dissociation of hemagglutinating and antibody measuring capacities of influenza virus, J Exper Med 85, 1-7.

Fraser, D, Mahler, H R, Shug, A L, and Thomas, C A, Jr, 1957, The infection of sub-cellular Escherichia coli, strain B, with a DNA preparation from T_2 bacteriophage, Proc Nat Acad Sc 43, 939-947

Friend, C, 1957, Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia, J Exper Med 105, 307-318

Frisch-Niggemeyer, W, and Hoyle, L, 1956, The nucleic acid and carbohydrate content of influenza virus A and of virus fractions produced by ether disintegration, J Hyg 54, 201-212

Gaylord, W H, Jr, and Melnick, J L, 1953, Intracellular forms of pox viruses as shown by the electron microscope (vaccinia, ectromelia, molluscum contagiosum), J Exper Med 98, 157-172

Gierer, A, and Schramm, G, 1956, Die Infektiositat der nucleinsaure aus tabakmosaicvirus, Ztschr Naturforsch 11b, 138-142

Gogolak, F M, and Ross, M R, 1955, The properties and chemical nature of the psittacosis virus hemagglutinin, Virology 1, 474-496

Goldfield, M, Srihongse, S, and Fox, J P, 1957, Hemagglutinins associated with certain human enteric viruses, Proc Soc Exper Biol & Med 96, 788-791

Gotlieb, T, and Hirst, G K, 1954, The experimental production of combination forms of virus III The formation of doubly antigenic particles from influenza A and B virus and a study of the ability of individual particles of X virus to yield two separate strains J Exper Med 99, 307-320

———, 1956, The experimental production of combination forms of virus VI Reactivation of influenza viruses after inactivation by ultraviolet light Virology 2, 235-248

Gottschalk, A, 1957, Virus enzymes and virus templates, Physiol Rev 37, 66-83

Groman, N B, 1955, Evidence for the active role of bacteriophage in the conversion of nontoxigenic

Corynebacterium diphtheriae to toxin production, J Bact 69, 9-15

Gross, L., 1955, Induction of parotid carcinomas and/or subcutaneous sarcomas in C_3H mice with normal C_3H organ extracts, Proc Soc Exper Biol & Med 88, 362-368

——, 1956, Viral (egg-borne) etiology of mouse leukemia Filtered extracts from leukemic C_{58} mice, causing leukemia (or parotid tumors) after inoculation into newborn C_{57} brown or C_3H mice Cancer 9, 778-791

Habel, K., Hornibrook, J W, Gregg, N C, Silverberg, R J, and Takemoto, K K., 1958, The effect of anticellular sera on virus multiplication in tissue culture, Virology 5, 7-29

Hallauer, C., 1946, Über den Virusnachweis mit dem Hirst-Test, Schweiz Ztschr f Path u Bakt 9, 553-554

Hamers-Casterman, C., and Jeener, R., 1957, An initial ribonuclease—sensitive phase in the multiplication of tobacco mosaic virus, Virology 3, 197-206

Hanig, M, 1948, Electrokinetic change in human erythrocytes during adsorption and elution of PR8 influenza virus, Proc Soc Exper Biol & Med 68, 385-392

Henle, G., Girardi, A., and Henle, W., 1955, A nontransmissible cytopathogenic effect of influenza virus in tissue culture accompanied by formation of noninfectious hemagglutinins, J Exper Med 101, 25-41

Henle, W., Liu, O C., Paucker, K., and Lief F S., 1956, Studies on host-virus interactions in the chick embryo influenza virus system XIV The relation between tissue-bound and liberated virus materials under various conditions of infection J Exper Med 103, 799-822

d'Herelle, F., 1917, Sur un microbe invisible antagoniste des bacilles dysentériques, Compt rend Acad sc 65, 373-375

l'Heritier, Ph., 1958, The hereditary virus of drosophila in Advances in Virus Research, vol 5 pp 195-245, New York, Acad Press

Hershey, A D., and Chase, M, 1951, Genetic recombination and heterozygosis in bacteriophage, Cold Spring Harbor Symp Quant Biol 16, 471-479

——, 1952, Independent functions of viral protein and nucleic acid in growth of bacteriophage, J Gen Physiol 36, 39-56

Hershey, A D, Kalmanson, G., and Bronfenbrenner, J., 1943, Quantitative relationships in the phage-antiphage reaction Unity and homogeneity of the reactants, J Immunol 46, 281-299

Hershey, A D., and Rotman, R., 1949, Genetic recombination between host-range and plaque-type mutants of bacteriophage in single bacterial cells, Genetics 34, 44-71

Hilleman, M R., Haig, D A., and Helmold, R J., 1951, The indirect complement fixation, hemagglutination and conglutinating complement absorption tests for viruses of the psittacosis-lymphogranuloma venereum group, J Immunol 66, 115-130

Hirst, [illegible] K., 1941, The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus, Science 94, 22-23

——, 1950, Receptor destruction by viruses of the mumps-NDV-influenza group, J Exper Med 91, 161-175

Horsfall, F L Jr 1954, On the reproduction of influenza virus Quantitative studies with procedures which enumerate infective and hemagglutinating virus particles J Exper Med 100, 135-161

——, 1955, Reproduction of influenza viruses Quantitative investigations with particle enumeration procedures on the dynamics of influenza A and B virus reproduction J Exper Med 102, 441-473

Horsfall, F L, Jr, and Hahn, R G 1940, A latent virus in normal mice capable of producing pneumonia in its natural host, J Exper Med. 71, 391-408

Howe C, Rose, [illegible] M and Schneider, L., 1957, Enzymic action of influenza virus on human erythrocyte stroma components Proc Soc Exper Biol & Med 96, 88-94

Hull, [illegible] N, Minner, J R., and Smith, J W, 1956 New viral agents recovered from tissue cultures of monkey kidney cells, Am J Hyg 63, 204-215

Huseby R A, Smith, F W, and Bittner, J J 1946, The genetic control of ovarian secretion as related to the incidence of mammary cancer in mice, Cancer Res 6, 494

Isaacs, A, 1957, Particle counts and infectivity titrations for animal viruses in Advances in Virus Research, vol 4, pp 111-158, New York, Acad Press

Iwanowski, D, 1892, Über die Mosaikkrankheit der Tabakspflanze Bull de l'Acad Imp des Sci de St Petersbourg, 3 n.s 67-70

Jacob F, and Wollman E L, 1956 Recherches sur les bactéries lysogènes défectives I Déterminisme génétique de la morphogenèse chez un bactériophage tempéré Ann Inst Pasteur 90, 282-302

Jeener R, 1956, Ribonucleic acids and virus multiplication, Advances Enzymol 17, 477-498

Jensen, [illegible] E, 1957, The nature of serological relationships among influenza viruses in Advances in Virus Research, vol 4, pp 279 310, New York, Acad Press

Jerne, N K., and Avegno, P., 1956 The development of the phage-inactivating properties of serum during the course of specific immunization of an animal Reversible and irreversible inactivation J Immunol 76, 200-208

Kalmanson, G M, and Bronfenbrenner, J, 1943, Restoration of activity of neutralized biologic agents by removal of the antibody with papain, J Immunol 47, 387-407

Kaplan, A S, 1955, The susceptibility of monkey kidney cells to poliovirus in vivo and in vitro, Virology 1, 377-392

Kellenberger, E, and Sechaud, S, 1957, Electron microscopical studies of phage multiplication II Production of phage-related structures during multiplication of phages T_2 and T_4 Virology 3, 256-274

Kidd, J G, and Rous, P, 1940, Cancers deriving from the virus papillomas of wild rabbits under natural conditions, J Exper Med *71*, 469-494

Kozloff, L M, Lute, M, and Henderson, K, 1957, Viral invasion I, II, III J Biol Chem *228*, 511-546

Lahelle, O, 1958, Capacity of certain Echo virus 6 strains to cause hemagglutination Preparation of Echo antisera Virology *5*, 110-119

Lahelle, O, and Horsfall, F L, Jr, 1949, Hemagglutination with GDVII strain of mouse encephalomyelitis virus Proc Soc Exper Biol & Med *71*, 713-718

Lanni, F, and Lanni, Y T, 1953, Antigenic structure of bacteriophage, Cold Spring Harbor Symp Quant Biol *18*, 159-168

Le Bouvier, G L, Schwerdt, C E, and Schaffer, F L, 1957, Specific precipitates in agar with purified poliovirus, Virology *4*, 590-593

Lebrun, J, 1956, Cellular localization of herpes simplex virus by means of fluorescent antibody, Virology *2*, 496-510.

Le Clerc, J, 1956, Action of ribonuclease on the multiplication of the influenza virus, Nature, London *177*, 578-579

Lederberg, E M, and Lederberg, J, 1953, Genetic studies of hypogenicity in E coli, Genetics *38*, 51-64

Levens, J H, and Enders, J. F, 1945, The hemoagglutinative properties of amniotic fluid from embryonated eggs infected with mumps virus, Science *102*, 117-120

Levine, S, Puck, T T, and Sagik, B P, 1953, An absolute method for assay of virus hemagglutinins, J Exper Med *98*, 521-531

Levine, S, and Sagik, B P, 1956, The interactions of Newcastle disease virus (NDV) with chick embryo tissue culture cells Attachment and growth Virology *2*, 57-68

Levinthal, C, and Fisher, H, 1952, The structural development of a bacterial virus, Biochim et biophys acta *9*, 419-429

Lucke, B, 1938, Carcinoma in the leopard frog Its probable causation by a virus, J Exper Med *68*, 457-468

Luria, S E, 1953, General Virology, New York, John Wiley & Sons 427 pp

———, 1958, The reproduction of viruses *in* Protoplasmalogia vol 4, Wien Springer (in press)

Luria, S E, and Human, M L, 1950, Chromatin staining of bacteria during bacteriophage infection, J Bact *59*, 551-560

Lush, D, 1943, The chick red cell agglutination test with the viruses of Newcastle disease and fowl plague, J Comp Path *53*, 157-160

Lwoff, A, 1953, Lysogeny, Bact Rev *17*, 269-337

Lwoff, A, Dulbecco, R., Vogt, M, and Lwoff, M, 1955, Kinetics of the release of poliomyelitis virus from cells, Virology, *1*, 128-139

McClelland, L, and Hare, R, 1941, The adsorption of influenza virus by red cells and a new in vitro method of measuring antibodies for influenza virus, Canad Pub Health J *32*, 530-538

McKee, A P, and Hale, W M, 1946, Reactivation of overneutralized mixtures of influenza virus and antibody, J Immunol. *54*, 233-243.

Manaker, R A, and Groupé, V, 1956, Discrete foci of altered chicken embryo cells associated with Rous sarcoma virus in tissue culture, Virology *2*, 838-840

Mandel, B, and Racker, E, 1953, Inhibition of Theiler's encephalomyelitis virus (GDVII strain) of mice by an intestinal mucopolysaccharide, J Exper Med *98*, 399-415

Meneghini, M, and Delwiche, C C, 1951, The multiplication of tobacco mosaic virus in the host plant, J. Biol Chem *189*, 177-186

Merrill, M H., 1936, The mass factor in immunological studies upon viruses, J Immunol *30*, 169-184

Mills, K C, and Dochez, A R, 1944, Specific agglutination of murine erythrocytes by a pneumonitis virus in mice, Proc Soc Exper Biol & Med *57*, 140-143

Mogabgab, W J, Green, I J, and Dierkhising, O C, 1954, Primary isolation and propagation of influenza virus in cultures of human embryonic renal tissue, Science *120*, 320-321

Morgan, C, Godman, G C, Rose, H M, Howe, C, and Huang, J S, 1957, Electron microscopic and histochemical studies of an unusual crystalline protein occurring in cells infected by type 5 adenovirus Preliminary observations J Biophys & Biochem Cytol *3*, 505-508

Morgan, C., Rose, H M, and Moore, D H, 1956, Structure and development of viruses observed in the electron microscope. III Influenza virus J Exper Med *104*, 171-182

Morgan, C, Solon, A E, Rose, H M, and Moore, D H, 1954, Structure and development of viruses observed in the electron microscope II Vaccinia and fowl pox viruses J Exper Med *100*, 301-310

Murphy, J S, 1957, A phage-associated enzyme of bacillus megaterium which destroys the bacterial cell wall, Virology *4*, 563-581

Murray, W S, and Little, C C, 1936, Extrachromosomal influence in relation to incidence of mammary and non-mammary tumors in mice, Am J Cancer *27*, 516-518

Nagler, F P O, 1942, Application of Hirst's phenomenon to titration of vaccinia virus and vaccinia immune serum, M J Australia *1*, 281-283

North, E A, 1944, A study of the immunological reactions of the variola and vaccinia viruses grown in the developing egg, Australian J Exper Biol & M Sc *22*, 105-109

Noyes, W F, and Mellors, R C, 1957, Fluorescent antibody detection of the antigens of the Shope papilloma virus in the papillomas of the wild and domestic rabbit, J Exper Med *106*, 555-562

Olitsky, P K, 1940, A transmissible agent (Theiler's virus) in the intestines of normal mice, J Exper Med *72*, 113-127

Olitsky, P K, and Yager, R H, 1949, Hemagglutination by Columbia SK, Columbia MM, mengo encephalomyelitis and encephalomyocarditis viruses experiments with other viruses, Proc Soc Exper Biol & Med *71*, 719-724

Prince, A M, 1958, Quantitative studies on Rous sarcoma virus III Virus multiplication and cellular response following infection of the chorioallantoic membrane of the chick embryo Virology 5, 435-457

Quersin Thiry, L, 1955, Action de serums antitissulaires sur certaines infections à virus Inst Pasteur de Bruxelles edit J Duculot, S A Gembloux.

Rivers, T. M, and Tillett, W S, 1924, Further observations on the phenomena encountered in attempting to transmit varicella to rabbits, J Exper Med 39, 777-802.

Robbins, F C, Enders, J F, Weller, T H, and Florentino, G L, 1951, Studies on the cultivation of poliomyelitis viruses in tissue culture V The direct isolation and serologic identification of virus strains in tissue culture from patients with nonparalytic and paralytic poliomyelitis Am J Hyg 54, 286-293

Rosen, L, 1958, Hemagglutination by adenoviruses, Virology 5, 577-580

Rous, P, 1911, Transmission of a malignant new growth by means of a cell free filtrate, J A M A 56, 198

Rous, P, and Beard, J W, 1935, The progression to carcinoma of virus-induced rabbit papillomas (Shope), J Exper Med 62, 523-548

Rous, P, Kidd, J G, and Smith, W E, 1952, Experiments on the cause of the rabbit carcinomas derived from virus-induced papillomas II Loss by the Vx2 carcinoma of the power to immunize hosts against the papilloma virus J Exper Med 96, 159-174

Rowe, W P, Huebner, R J, Gilmore, L K, Parrott, R H, and Ward, T G, 1953, Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture Proc Soc Exper Biol & Med 84, 570 573

Rubin, H, 1955, Quantitative relations between causative virus and cell in the Rous No 1 chicken sarcoma Virology 1, 445-473

———, 1957, Interactions between Newcastle disease virus (NDV), antibody and cell, Virology 4, 533-562

Rubin, H, and Franklin, R M, 1957, On the mechanism of Newcastle disease virus neutralization by immune serum, Virology 3, 84-95

Rubin, H, Franklin, R M, and Baluda, M, 1957, Infection and growth of Newcastle disease virus (NDV) in cultures of chick embryo lung epithelium, Virology 3, 587-600

Sabin, A B, and Buescher, E L, 1950, Unique physico-chemical properties of Japanese B encephalitis virus hemagglutinin, Proc Soc Exper Biol & Med 74, 222 230

Sagik, B, Puck, T, and Levine, S, 1954, Quantitative aspects of the spontaneous elution of influenza virus from red cells, J Exper Med 99, 251-260

Schafer, W, 1957, Units isolated after splitting fowl plague virus in Ciba Foundation Symp, London, Churchill, pp 91-103

Schlesinger, R W, 1950, Incomplete growth cycle of influenza virus in mouse brain, Proc. Soc Exper Biol & Med 74, 541-545

Schramm, G, 1947, Über die Spaltung des Tabakmosaikvirus und die Wiedervereinigung der Spaltstücke zu Höhermolekularen Proteinen Ztschr Naturforsch 2b, 112-121, 249-257

Schwerdt, C E, and Schaffer, F L, 1956, Purification of poliomyelitis viruses propagated in tissue culture, Virology 2, 665-678

Shope, R E, 1932, A filtrable virus causing a tumor-like condition in rabbits and its relationship to virus myxomatosum, J Exper Med 56, 803-822

———, 1933, Infectious papillomatosis of rabbits, J Exper Med 58, 607-624

———, 1943, Swine influenza in Virus Diseases, pp 85-109, Ithaca, Cornell Univ Press

Siegel, A, and Wildman, S G, 1956, The inactivation of infectious centers of tobacco mosaic virus by ultraviolet light, Virology 2, 69-82

Smith, W, Andrewes, C H, and Laidlaw, P P, 1933, A virus obtained from influenza patients, Lancet II, 66-68

Sonneborn, T M, 1949, Beyond the gene, Am Sc 37, 33-59

Sprunt, K, Mountain, I M, Redman, W M, and Alexander, H E, 1955, Production of poliomyelitis virus with combined antigenic characteristics of type I and type II Virology 1, 236-249

Stanley, W M, 1943, Chemical structure and the mutation of viruses in Virus Diseases, pp 35-59, Ithaca, Cornell Univ Press

Stent, G S, and Wollman, E L, 1950, Studies on activation of T_4 bacteriophage by cofactor II Mechanism of activation Biochim et biophys acta 6, 307-316

Stewart, S E, Eddy, B E, Gochenour, A M, Borgese, N G, and Grubbs, G E, 1957, The induction of neoplasms with a substance released from mouse tumors by tissue culture, Virology 3, 380-400

Stone, J D, 1946, Inactivation of vaccinia and ectromelia virus haemagglutinins by lecithinase, Australian J Exper. Biol & M Sc 24, 191-196

———, 1948, Prevention of virus infection with enzyme of V cholerae I and II Australian J Exper Biol & M Sc 26, 49-64, 287-298

Stone, J D, and Ada, G L, 1950, Electrophoretic studies of virus-red cell interaction Additive effect of viruses of the influenza group and the receptor-destroying enzyme of V cholerae, Brit J Exper Path 31, 275-284

———, 1952, Electrophoretic studies of virus-red cell interaction Relationship between agglutinability and electrophoretic mobility Brit J Exper Path 33, 428-439

Stone, J D, and Burnet, F M 1946, The production of vaccinia haemagglutinin in rabbit skin, Australian J Exper Biol & M Sc 24, 9-13

Streisinger, G, 1956a, Genetic control of host range and serological specificity in bacteriophages T_2 and T_4, Virology 2, 377-387.

———, 1956b, Phenotypic mixing of host range and serological specificities in bacteriophages T_2 and T_4, Virology 2, 388-398

Tamm, I, and Horsfall, F L, Jr, 1950, Characterization and separation of an inhibitor of viral hemagglutination present in urine, Proc Soc Exper Biol & Med *74*, 108-114

Tolmach, L J, 1957, Attachment and penetration of cells by viruses *in* Advances in Virus Research, vol 4, pp 63-110, New York, Acad Press

Traub, E, 1939, Epidemiology of lymphocytic choriomeningitis in a mouse stock observed for four years, J Exper Med *69*, 801-817

Twort, F W, 1915, An investigation on the nature of ultra-microscopic viruses, Lancet *189*, 1241-1243.

Valentine, R C, and Isaacs, A, 1957a, The structure of influenza virus filaments and spheres, J Gen Microbiol *16*, 195-204

———, 1957b, The structure of viruses of the Newcastle disease-mumps-influenza (myxovirus) group, J Gen Microbiol *16*, 680-685

Verlinde, J D, de Baan, P, Kret, A, and Waller-Fetter, P, 1951, *Studies on the hemagglutination* by the EMC-MM-Columbia SK-group of viruses III Destruction of virus receptors *in vitro* and *in vivo* Antonie v Leeuwenhoek *17*, 137-145

Visconti, N, and Delbruck, M, 1953, The mechanism of genetic recombination in phage, Genetics *38*, 5-33

Vogt, M, and Dulbecco, R, 1958, Properties of a HeLa cell culture with increased resistance to poliomyelitis virus Virology *5*, 425-434

von Magnus, H, Gear, J H S, and Paul, J R, 1955, A recent definition of poliomyelitis viruses, Virology *1*, 185-189

von Magnus, P, 1954, Incomplete forms of influenza virus *in* Advances in Virus Research, vol 2, pp 59-79, New York, Acad Press

Walker, D L, and Horsfall, F. L, Jr, 1950, Lack of identity in neutralizing and hemagglutination-inhibiting antibodies against influenza viruses, J Exper Med. *91*, 65-86

Weidel, W, and Primosigh, J, 1957, Die gemeinsame Wurzel der Lyse von *Escherichia coli* durch Penicillin oder durch Phagen, Ztschr Naturforsch *12b*, 421-427

Weller, T. H., Robbins, F C., and Enders, J. F., 1949, Cultivation of poliomyelitis virus in cultures of human foreskin and embryonic tissues, Proc Soc Exper Biol & Med. *72*, 153-155

Williams, R C, and Smith, K M., 1957, A crystallizable insect virus, Nature, London *179*, 119-120

Woodruff, A M, and Goodpasture, E W, 1931, The susceptibility of the chorio-allantoic membrane of chick embryos to infection with the fowl-pox virus Am J Path *7*, 209-222

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5

Interference

INTRODUCTION

In the course of laboratory investigations of different virus diseases, situations inevitably arose in which a host was exposed to simultaneous infection with two viruses In several instances, accidental or deliberately contrived, it was found that infection with one virus mitigated the severity of disease produced by the other This phenomenon, unrelated to conventional immunologic reactions, bore superficial resemblance to certain types of cross-protection in mixed bacterial and parasitic infections of animals and was thought to be due to mutual "interference" between the two viruses involved

Because of the intimate dependence of viruses on the susceptible cell as a medium for propagation, it was assumed that interfering and suppressed viruses somehow competed for the same cells, and that a cell could not support the simultaneous replication of two viruses. This simple interpretation did not stand up under experimental test Instead, it has been found that concurrent infection of a host or even a single cell with two different viruses can express itself in a number of ways (1) both viruses multiply side by side and produce independently characteristic manifestations of infection (*dual infection*), (2) the viruses undergo some sort of genetic interaction and produce progeny with new properties (*recombination*), (3) one virus so modifies cells or the tissues of a host that a second one cannot multiply normally or cannot produce characteristic injury (*interference*) In addition, some effects have been reported, such as "exaltation," which require further clarification before they can be characterized within or outside of this framework. These various relationships can be demonstrated in mixed infection not only with two unrelated viruses but also with different mutants of the same virus, provided that the infecting dose is large enough to permit invasion of single cells by more than one particle. Altogether, quantitative factors are of paramount importance in determining whether a given set of circumstances will lead to the production of interference or alternate manifestations of mixed infection

EXPRESSIONS OF INTERFERENCE

Interference may be defined by two parameters: (1) inhibition of multiplication of one virus in a host system simultaneously infected with another virus, (2) suppression of injury, disease, or death due to one virus by simultaneous infection with another. From the point of view of possible medical significance, the latter is perhaps of more immediate interest In experimental systems, however, inhibition of viral multiplication is often the only observable effect, and it is on such systems that mechanisms can best be studied.

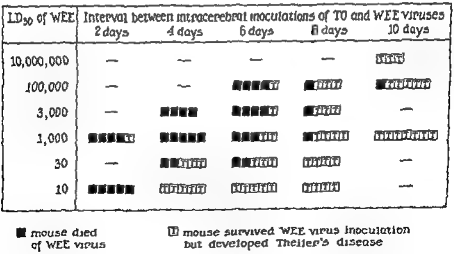
INTERFERENCE WITH DISEASE PRODUCTION IN EXPERIMENTAL ANIMALS

Historically, it was the suppression of disease or death in already infected animals

which first called attention to the existence of a mechanism of host resistance to viral infection other than classic humoral or nonspecific forms of immunity. Although occasional hints at interferencelike cross-protection can be traced to early clinical empiricism or experimental work, clear recognition of the specialized aspects of interference has come relatively recently For a comprehensive review of the subject see Henle (1950) The following examples have been selected arbitrarily to illustrate the diversity of experimental systems involving interference

Interference Between Unrelated Viruses. In 1937, Dalldorf, Douglass and Robinson reported that monkeys infected with lymphocytic choriomeningitis virus failed to become paralyzed when superinfected with poliovirus This type of protection lasted about 2 weeks, and after 1 month full susceptibility to poliovirus was restored Because of the transitory nature of resistance and because the 2 agents were not antigenically related, this sparing effect was considered to be a new immunity mechanism in the virus field Dalldorf et al also observed that spinal cords of doubly infected monkeys did not contain as much virus as those of polio-infected control animals.

Subsequently, similar observations were made with a variety of other viral combinations The dependence on timing and dosage of the effectiveness of interference was illustrated by experiments with the TO strain of Theiler's virus (*poliovirus muris*) and Western equine encephalomyelitis (WEE) virus (Schlesinger et al , 1943) Intracerebral inoculation of the TO virus induces in mice, after an incubation period of about 2 weeks, a paralytic disease of the poliomyelitis type. The virus multiplies to a titer of about $10^{3.5}$ ID_{50} WEE virus, on the other hand, causes a rapidly fatal form of diffuse encephalomyelitis and attains titers of the order of 10^{9} LD_{50} Figure 42 illustrates what happens when mice are infected with both viruses If TO virus is injected intracerebrally immediately before or together with minimal amounts of WEE virus, all mice succumb to the latter. If TO virus is given first and followed after increasing intervals by WEE virus, progressively larger amounts of the latter are required to bring about infection At 10 days, the mice resist as much as 10



experiment)

million lethal doses of the far more virulent equine virus. Even after inoculation of such excessive doses, no multiplication of this virus is demonstrable. In contrast, the course of Theiler's disease is not affected by the interposed inoculation of WEE virus, and the increasing protection can be correlated directly with the multiplication and the progressive spread of TO virus within the central nervous system.

In other systems it has been found that the interfering virus need not be pathogenic or capable of unrestricted multiplication. Multiplication of egg-adapted strains of influenza virus in mouse brain is known to be restricted to a single cycle yielding abnormal, noninfectious virus. Vilches and Hirst (1947) have shown that such strains, inoculated intracerebrally, nevertheless protect mice against the lethal effects of various encephalitic viruses, e.g., WEE virus. In this case, the interfering agent has to be given either within 2 weeks before or together with the suppressed virus. Since the interfering infection is not progressive, effective interference depends on initial administration of massive doses of influenza virus.

The same requirement for saturating doses applies in those cases in which successful interference with virulent agents is exerted by *inactivated* viruses. For example, Vilches and Hirst (1946) have found that ultraviolet-irradiated influenza virus retains at least part of its capacity to protect mice against WEE virus. The effect of inactivation will be discussed more fully in a later section.

Interference by Avirulent with Virulent Variants of the Same or Related Viruses. Results obtained when immunologically related viral pairs are inoculated together or consecutively in animals are much more difficult to interpret as interference than the previously described examples, because the operation of specific humoral immunity mechanisms must be ruled out. The observation by Hoskins (1935) of protection against viscerotropic yellow fever virus by simultaneous inoculation of a neurotropic variant is a case in point. the strongest argument against the role of antibody in this system stemmed from the later finding (Findlay and MacCallum, 1937) that neurotropic yellow fever virus similarly protected against the serologically distinct Rift Valley fever virus.

A classic observation by Magrassi (1935) concerned the competition (*Konkurrenzphänomen*) between 2 doses of herpes simplex virus given by different routes of inoculation. For example, subcutaneous injection of herpes virus in the abdominal wall led to ascending myelo-encephalitis with death, usually in the second week. Intracerebral infection, in contrast, killed rabbits after 3 to 4 days. When intracerebral reinfection followed primary subcutaneous inoculation by about 7 days, animals were protected—the 2 lethal doses had canceled each other out. This was not effective with either shorter or longer intervals. The protection seemed to be strictly limited both in area and in time. This example and a number of modifications reported by Magrassi and later by Doerr and co-workers seem to illustrate a combination of interference and of local, specific immune mechanisms.

An interesting manifestation of interference, and one of importance in connection with adaptation of viruses to new experimental host systems, has come to the fore in recent years with the increased use of mice for attenuation of viruses by intracerebral serial passages. In the course of such passages, virulence for the mouse (neurotropism) may develop gradually, presumably as a result of multiple mutational steps and of selective advantage of the virulent mutants. When unadapted or partially adapted viruses are titrated in mice the mortality rate is often higher among those inoculated with small than with large doses of viruses. Such paradoxical titrations have been seen in work with several viruses, e.g., yellow fever, Rift Valley fever and dengue viruses. In the last case, it was found that viral populations giving paradoxical effects on titration were mixtures of avirulent and virulent particles in proportions permitting the former to block the latter at high, but not low, concentrations (Schlesinger, Frankel, and Winter, to be published). This type of phenomenon is called "auto-interference."

Interference with Cytopathogenic Effects in Tissue Cultures

The development of tissue culture systems suitable for quantitative study of viruses has made possible the demonstration of interference at the cellular level divorced from immune mechanisms of intact host animals

Qualitatively, interference in metabolizing tissue fragments had been shown earlier for different strains of influenza virus (Andrewes, 1942) and for a variety of neurotropic viruses (Huang, 1943; Lennette and Koprowski, 1946). Indeed, Huang, in his studies on interference, used the metabolic inhibition which was reintroduced later for extensive use in neutralization tests with poliovirus. He showed that cell destruction by certain viruses (e g., WEE) could be detected by adding an indicator dye to the liquid medium. Normally metabolizing tissue produced acid with a resulting color change, while destructive viruses prevented such a change Using this criterion, he was able to demonstrate interference between nondestructive (St Louis encephalitis) and destructive (WEE) viruses

Recognition of specific cytopathogenic effects as an expression of virulence, and introduction of the plaque assay technic for quantitation of animal viruses have significantly refined work on the mechanism and the dynamics of interference. Various studies utilizing these phenomena for analyses of interference with homologous or heterologous viral combinations have shown that the protective function of an interfering virus, whether active or inactivated, can be demonstrated in tissue cultures as convincingly as in animals Some of these investigations which have furthered our knowledge on the general characteristics and mechanisms of interference will be discussed more fully in the appropriate later sections

Interference with Multiplication of Influenza Virus in the Chorio-allantoic Membrane of Chick Embryos (CAM)

Use of the influenza virus-CAM system as a model for work on fundamental aspects of viral infections offers these advantages: (1) viral multiplication can be demonstrated and quantitated even in absence of death or injury to the host by measuring titers of viral hemagglutinin (HA) or complement-fixing antigens (S or V antigen); (2) the viral yield can be determined quantitatively as well as qualitatively in terms of its infectious titer relative to its HA titer (ID/HA ratio), with low ratios indicating preponderance of noninfectious hemagglutinating (incomplete) virus; (3) virus is liberated by infected cells into the allantoic fluid (AF), and thus separation of extracellular viral particles from virus associated with cells is facilitated; (4) this system can be improved still further by deembryonation, which eliminates all contents of the egg other than the CAM, or by maintenance of surviving CAM fragments in defined media; (5) the role of viral enzyme and its mucoprotein substrate (cellular receptors) in adsorption of viral particles has been established, and adsorption can be blocked by receptor-destroying enzyme (RDE of *V. cholerae*); (6) interaction of strains (interference, dual infection, genetic combination) can be investigated because multiple genetic markers are available for easy identification of yields (e g , serologic specificity, virulence for mice or chick embryos, enzymatic activity, heat stability)

Application of these technical aids to investigations on interference has yielded many important findings, especially with regard to the fate of interfering and suppressed virus Mutual interference between active A and B strains of influenza virus was demonstrated in 1944 by Ziegler and Horsfall. When relatively small amounts of each type were inoculated simultaneously, both multiplied When one had a head start of 8 to 12 hours, the other one was suppressed, unless it was given in excessively large doses

Subsequent experiments by Burnet and Hirst and their co-workers on genetic recombination between different strains of influenza virus have confirmed that one strain of virus, given the proper advantage, can block multiplication of another This works for heterotypic (A vs. B) as well as for homotypic pairs distinguishable by strain-specific antigens or various other attributes. On the other hand, when the same pairs are inoculated in equivalent amounts, the progeny may include many viral particles containing genetic or nongenetic markers contributed by both parent strains. Since such genetically or phenotypically mixed particles must arise in cells in-

fected with both parent strains, it is clear that interference between 2 active influenza viruses at the cellular level is not an absolute all-or-none phenomenon, but that it occurs only under special conditions

The situation is somewhat more clear-cut when *inactivated* virus is used as the interfering agent. It was first reported by Henle and Henle (1943) and Ziegler et al (1944) that ultraviolet-irradiated virus, when inoculated in large doses into the allantoic cavity, inhibited the propagation of active influenza virus of either the homologous or the heterologous type Inhibition occurred when the irradiated virus was given simultaneously with or as long as 96 hours (longest interval tested) before inoculation of the active virus Interference was obtained even when the inactivated virus was given shortly *after* the active virus, *i e*, *before newly produced progeny* of the latter had appeared Under these conditions, homotypic irradiated virus completely inhibited multiplication, while heterotypic virus permitted it to proceed in those cells already infected with active virus By the use of heterotypic irradiated virus it was possible to obtain one-step growth curves for influenza viruses (Henle et al, 1947) That is to say that spread of newly produced virus to primarily uninfected cells was prevented by the blocking action of the inactivated virus

The Relation of Incomplete Virus Production to Interference

Another basic observation concerns the demonstration of auto-interference in eggs inoculated with large amounts of influenza virus This manifests itself in the form of release of noninfectious hemagglutinating virus (incomplete virus) Under standard conditions, when eggs are inoculated with dilute viral suspensions (e g, 10^{-4} diluted infected AF containing 100 to 1,000 ID_{50}), the viral progeny harvested at about 40 hours has an ID/HA ratio of around $10^{6}/1$ to $10^{6.5}/1$ * Such standard viral populations contain, on the average, about 10 virus particles per infectious unit, as determined by electron microscopic particles count, HA titrations and chemical analysis (Isaacs, 1957). Hence, by definition, 9 of every 10 viral particles may be classified as noninfectious. The proportion of noninfectious virus is greatly increased in AF harvested from eggs inoculated with undiluted standard virus containing more than 10^{5} ID_{50} Horsfall (1954) and Paucker and Henle (1955) have shown that this phenomenon can be further enhanced by using as seed inoculum virus which has been heated at 37° C. for several days and thus has acquired a much reduced *ID/HA* ratio.

Von Magnus reported in 1951 that serial egg-to-egg transfer of undiluted infected AF (*undiluted passage series*) yielded virus of increasing incompleteness, i e., decreasing ID/HA ratio The lowest ratio of 10/1 to 100/1 was reached in the third or fourth undiluted passage, this ratio indicating that only about 0.00001 to 0 000001 per cent of the viral progeny was infectious HA titers in such yields rose to normal or nearly normal levels, suggesting that viral propagation as such was not suppressed. Nevertheless, this progressive qualitative change in properties of the virus has been interpreted as interference with production or maturation of normal virus resulting from overloading of cells at the time of infection While it seems reasonable to postulate that the low yield of infectious virus may be due to interference by noninfectious with infectious virus, the role of this mechanism in the genesis of the incomplete virus itself remains problematical For example, it has been reported that incomplete virus can be obtained in eggs infected with relatively small doses of virus, and the same may be true for another host tissue, viz, mouse brain, in which the multiplication of certain influenza strains is restricted to a single cycle yielding incomplete virus (Schlesinger, 1950, 1953) The subject is discussed in further detail in Chapter 4

GENERAL CHARACTERISTICS AND INTERPRETATION OF INTERFERENCE PHENOMENA

Interference as a Localized Phenomenon. Critical quantitative findings in tissue cultures have pinpointed the individual cell as the site of the phenomenon. This conclusion is in line with earlier studies which have clearly shown that protection by interference is not associated with a systemic change in

* It should be pointed out that this ratio holds true only if the HA titration is carried out under standardized conditions with approximately $10^{7.2}$ RBC per agglutination tube Reduction in the number of RBC would lead to a corresponding increase in HA titer, since the titer is that dilution of virus at which the number of viral particles roughly equals the number of RBC (Isaacs, 1957)

the intact host's response to the suppressed virus, but that it is limited to those tissues or cells which have had contact with the interfering agent Thus, when an interfering dose of ultraviolet (UV) irradiated influenza virus is inoculated into the allantoic cavity of embryonated eggs, the amniotic sac retains *susceptibility to infection with active virus* (Henle, Henle, and Kirber, 1947) The same principle probably applies to several observations in which protection against neurotropic viruses was limited to the neural pathway already traveled by another virus

Nature of Interfering Agents The interfering capacity has always been found to be inseparable from the viral particles themselves, except for a very recent observation suggesting the liberation of a nonviral interfering compound from cells exposed to inactivated influenza virus (see below, *Mechanisms of Interference*) *If the interfering* virus is noninfectious in the sense that it is incapable of detectable multiplication, it nevertheless contains specific antigens or possesses some other viral properties such as, in the case of myxoviruses, the ability to agglutinate erythrocytes That the interfering capacity is independent of reproductive capacity is illustrated by the fact that UV- or heat-inactivated *myxoviruses are potent interfering* agents In some instances, the interfering virus is restricted in its multiplication either because it is not adapted to the particular host system (e g, influenza virus in mouse brain) or because of the intervention of other factors, such as specific humoral immunity All available evidence indicates that effective interference depends on the native or residual ability *of the virus to interact in* some specific manner with the host cells Powell and Pollard (1956) have examined the effect of ionizing and monochromatic UV irradiation on the interfering property of influenza virus Their data suggest that the interfering capacity resides in a very small portion of the viral particle corresponding to a unit of about 2×10^6 A^3 in volume and molecular weight of *1,600,000 The action spectrum suggests* that the active moiety may be protein associated with the viral RNA, but a possible role of lipids has not been ruled out

***Time and Dosage Factors** Regardless* of its nature or of the particular way in which its interaction with the host cells manifests itself, the interfering virus, in order to be successful, must have a quantitative advantage or a head start over the virus that is to be suppressed Hence, if a mixture containing equal amounts of 2 viruses is inoculated into a host, chances are that the one which infects or multiplies more rapidly will predominate in the yield; or, if in a mixture one virus exceeds the other in amount, it will as a rule act as the interfering agent. By the same token, viruses which are restricted in their multiplication or have been inactivated must be administered in large doses in order to be effective as interfering agents In line with these general principles, the interfering virus must be given before, together with, or—other quantitative conditions being favorable—shortly after the virus which is to be suppressed

This intimate dependence on dosage and timing strongly suggests that the interfering *virus must saturate the susceptible cells and* must modify them in some manner. Thus, the number of cells lining the allantoic cavity has been variously estimated to total between $10^{7.2}$ and $10^{8.9}$, and maximally effective interference by *UV-inactivated with active influenza* virus in the CAM requires inoculation of about 10^8 to 10^9 viral particles. Even after such saturating doses of inactivated virus (multiplicity greater than 1 viral particle per cell) maximum interference is not established momentarily but only after some 16 hours (Fazekas de St. Groth et al, 1953; Paucker, 1958) During the intervening period, over-all viral multiplication is gradually retarded, and the yield is progressively lowered Kinetic data also suggest that in a certain proportion of the cells multiplication of superinfecting active virus is not blocked, no matter what the multiplicity of the inactivated virus

In this connection, the occurrence of genetically or phenotypically mixed progeny particles in eggs infected with different influenza strains clearly shows that the mere association of one viral particle with a cell [illegible]

certain proportion of the cells may yield recombinant progeny particles, indicating that the in[illegible]
ply by [illegible]
agent [illegible]
ent conflict between the concepts of interfer-

ence and of dual infection resolves itself if it is appreciated that viral populations, especially after partial inactivation by physical means, are undoubtedly inhomogeneous with regard to various biologic properties

Allowing for these qualifications, it can be concluded that interference by inactivated influenza virus may be induced in a single cell by effective combination with a single viral particle. This conclusion has received convincing experimental backing from studies by Baluda (1957) on the capacity of UV-irradiated Newcastle disease virus (NDV) to interfere with active NDV in monolayers of chick embryo lung cells In this system, the number of cells and the number of inactivated and of superinfecting active viral particles can be determined accurately, and the total yield of virus as well as the yield per cell can be measured by the plaque assay method Baluda's findings indicate (1) that *a single inactive particle per cell can induce interference*, (2) that *interference may be established in some cells within a fraction of a minute* A certain proportion of the cells which have adsorbed UV-irradiated virus can be superinfected with active virus provided that the multiplicity of the latter is high enough

Sensitive tissue culture methods have also been applied by Levine (1957) to a quantitative analysis of the mutual interference between active myxoviruses and WEE virus This system apparently differs from others in principle in that production of NDV in single cells can be diminished by superinfection with WEE virus as long as 5 to 6 hours after infection with NDV. At the same time, the ability of NDV-infected cells to produce WEE virus decreases exponentially with time after primary NDV infection In other words, there is a period after primary infection with NDV, during which superinfection with WEE virus results in multiplication of both agents, but to lower titers than in singly infected cells.

MECHANISM OF INTERFERENCE

An examination of facts known about interference makes it abundantly clear that it is localized in the individual cell Obviously, an understanding of mechanisms requires far more basic knowledge about viral reproduction than we now have What is particularly vexing is the diversity of viral combinations with which interference can be demonstrated

A consistent pattern has emerged from investigations into the events leading from exposure of a cell to virus to the ultimate release of viral progeny This pattern involves: (1) *collision* between virus and cell, requiring proper electrostatic forces and absence from the extracellular environment of inhibitory substances; (2) specific *adsorption*, the mechanism of which is understood only in the case of myxoviruses, (3) *penetration* into the cell; (4) *disintegration or loss of identity* of infecting viral particles, (5) *eclipse* period; (6) appearance in increasing amounts of *newly produced viral materials*, including antigens, hemagglutinins and, finally, infectious virus; (7) *release* of newly produced virus and viral products by gradual secretion or upon disintegration of the cells These processes may or may not be associated with recognizable histologic changes Use of histochemical methods, including fluorescein-labeled antibody and of electron microscopy, and physical separation of cellular particulates has refined study at the cellular level, especially the distinction between "nuclear" and "cytoplasmic" viruses. An imposing body of evidence is accumulating in support of the essential role of viral nucleic acid, especially RNA, as the genetic material of many animal viruses and of the idea that genetic and nongenetic viral constituents are replicated separately Beginnings have been made in the development of antimetabolites which are thought to inhibit (or promote) viral multiplication through their effects on biosynthetic processes, especially those concerned with nucleoprotein metabolism (Chap 6). Finally, certain substances, notably bacterial endotoxins, have been found to inhibit viral toxicity without significantly influencing viral reproduction

These various facets are recapitulated here (for more comprehensive discussion see Chap 4), because the key to the mechanism of interference clearly lies in an understanding of the fate of the interfering and the suppressed virus, of their effects upon the cell, and of the specific viral constituents responsible for the observed activities. Only rudimentary beginnings have been made in these directions

Perhaps the simplest concept could explain those examples in which progressive infection with one pathogenic virus induces increasing protection against another (Fig 42), one could simply postulate that the interfering virus has led to general, nonspecific damage

to or death of so many cells that the *second virus finds itself without a suitable medium.* This explanation fails to do justice to those cases where interference does not occur or is induced by avirulent, nonmultiplying, or inactivated viruses Here the clues have to be sought in terms of more specific and subtle relationships

The demonstration of *dual infection of single cells* with viruses producing intranuclear (herpes simplex) and cytoplasmic (vaccinia) inclusions (Syverton and Berry, 1947) perhaps suggests that interference may not occur between viral pairs utilizing for their replication different cellular matrices. One may look in the same light upon the fact that certain virus-induced tumor cells support the propagation of superinfecting unrelated viruses better than normal tissues and cells of the tumor-bearing animals.

On the other hand, some viral pairs which do display one-way or reciprocal interference are so far apart in their general biologic properties that it is difficult to imagine common factors involved in their genesis (influenza or NDV vs WEE virus). Yet, the recent work by Levine (1957) has shown that two such viruses can compete with one another in the individual cell with the result that the yield of each is reduced This finding, coupled with reaffirmation of earlier indications that destruction of cellular mucoprotein receptors by myxoviruses or RDE has no effect on the infectability of cells by WEE virus, would suggest that the *competition* may be *for cellular constituents needed for the synthesis of both viruses.*

Such direct competition appears to be more unlikely in systems utilizing *nonmultiplying* or *inactivated* viruses as interfering agents We may ask, first, what is the *fate of the interfering virus?* The most searching studies have been done with UV- or heat-inactivated myxoviruses in eggs (Henle, 1950) and tissue culture (Baluda, 1957) UV-irradiated influenza virus is *adsorbed* by the allantoic cells, and after a short period of time its interfering action can no longer be neutralized by antibody or released from the cell by RDE: it has *entered the cell.* In contrast, attachment of UV-irradiated NDV to chick embryo lung *monolayers remains* partially reversible by anti-NDV antibody in that such treatment prevents exclusion of superinfecting active virus in about 50 per cent of the cells This observation by Baluda suggests that interference follows upon superficial attachment of inactivated particles to the cell The contrast between the two systems may be fundamental but may also be due to physiologic differences between allantoic cells in the intact egg and epithelial lung cells in monolayers. The extent to which inactivated viruses can enter into an association with host cells may be revealed in future studies, especially with radioisotope-labeled virus.

However, the *maintenance of refractoriness* to superinfecting active virus appears to depend on continued association of interfering virus with the cell. In the NDV system, this association breaks down after 26 to 60 hours, and then the cells' susceptibility to active NDV is restored. The effect of UV-inactivated *influenza virus is longer lasting—perhaps* again suggestive of a more profound integration of the virus into the cell It should be remembered that UV-irradiated influenza virus has been shown to be reactivable in allantoic cells by active virus of a different strain, as indicated by the fact that from such doubly infected cells genetically mixed progeny particles can be obtained This would support the idea that at least a fraction of the irradiated virus can go beyond the stage of superficial adsorption A benzimidazole derivative (2,5-dimethylbenzimidazole) which is thought to inhibit the multiplication of influenza virus through its effect on the nucleic acid metabolism has been reported to prevent interference by inactivated influenza virus (Tyrrell and Tamm, 1955). In line with this is the suggestion offered by Powell and Pollard (1956) on the basis of irradiation experiments that retention of interfering capacity may depend on the integrity of a minute component of the viral particle, perhaps one associated with viral RNA

A high degree of integration of interfering virus into the cell may exist in several cases in which a long-lasting carrier state has been induced in *tissue culture cells surviving infection* with active virus (poliovirus, WEE, virus, NDV) Such cells have been found to resist superinfection with homologous or unrelated viruses. *A revealing study of this sort* concerns persistent infection of HeLa cells

with NDV (Cieciura et al, 1957) In HeLa cell cultures exposed to active NDV, less than 0 1 per cent of the cells survived, and these were grown to mass cultures From these, individual cells were removed and used to establish pure clones Clones were grown over a period of 2 years through hundreds of cell generations. The cells appeared quite normal but retained resistance to superinfecting NDV. When they were seeded on X-irradiated feeder layers of cells, they released active NDV which destroyed both themselves and the feeder layer Thus, it was shown that the original infection had been carried over from cell to cell without recognizable damage Superficially, this system suggests an analogy to lysogeny in phage-infected bacteria (Chap 7), but more recent evidence suggests that the carried virus is neutralizable by antiviral serum and thus located near the cellular surface rather than present in the form of provirus At any rate, the finding of resistance of these cells to superinfection again points to the *dependence of interference on the continued association of the interfering agent with the host cell*

If little is known about the fate of interfering viruses, even less can be said about the manner in which the interfered cell disposes of the suppressed virus For the most thoroughly studied systems, those involving myxoviruses, it has been found that (1) no virus-inhibiting substances are liberated by the cells into their environment, and (2) adsorption of superinfecting virus is not prevented Baluda's studies (1957) on NDV in tissue culture pretreated with UV-inactivated NDV have demonstrated that superinfecting virus disappears more rapidly than from normal cells That fraction which remains attached can be inactivated by antibody at a time when virus in association with normal cells would not be affected by such treatment because it has penetrated into the cells It has been suggested on the basis of these findings that active virus, after adsorption on interfered cells, is either *inactivated at the cellular surface* or prevented from *penetrating into the cell*. A recent provocative finding by Isaacs et al (1957) promises to clarify this aspect further These authors have reported that surviving fragments of allantoic membrane of chick embryos, exposed to heat-inactivated influenza virus and refractory to infection with active virus, liberate into the medium a macromolecular material (Interferon) which in turn can block infection of normal allantoic cells to which it has been added Interferon has no recognizable viral properties, is not neutralized by antiviral immune serum and cannot withstand heating at 60° C. for 1 hour The mode of its liberation suggests that it is a specific product of (abortive?) viral activity in the cell, but its biologic and chemical nature requires further study. It does not inactivate virus directly but acts only in conjunction with cells

It has been mentioned already that the *modus operandi* in certain other systems may be connected with more advanced stages of the infectious cycle, i e, that inactivation at the cellular surface or prevention of penetration of superinfecting virus do not account for the observed effects (e g, the lowered yields of both agents in single cells infected with NDV and WEE virus) In systems in which an avirulent variant protects a host against a virulent variant of the same or a related virus, it may be difficult to distinguish between true interference (i e., inhibition of multiplication of the virulent component) and multiplication of both, resulting in genetic recombination and dominant expression of the avirulent character

These considerations make it clear that mechanisms involved in interference may be as diverse as are the experimental observations interpreted as interference The complexities of the virus-cell interaction leave room for speculative analogies to a broad spectrum of biologic phenomena which may explain why the study of interference between viruses is of such dominant interest to many workers in the field. For example, in considering inhibition of penetration or surface inactivation of superinfecting virus, one may think of the restriction of fertilization to a single sperm and exclusion of others Mutual interference has also been demonstrated between different DNA preparations in the transformation of bacteria Considering the profound metabolic derangements which must accompany synthesis of viral materials, both genetic and nongenetic, in the infected cell, it is likely that mechanisms similar to those which can block or *modify constitutive* or

adaptive biosynthetic processes may play a role in competition between viruses. Finally, there are certain obvious analogies of interference between animal viruses to similar phenomena observed with bacteriophages. The basic concepts formulated from work on bacterial viruses are discussed fully in Chapter 7. They are an important guide in the interpretation of interference between animal viruses.

MEDICAL IMPLICATIONS OF INTERFERENCE

As long as relatively few viruses were known as human pathogens, and each disease they caused seemed to be either a self-limiting or a fatal episode, interference could be regarded chiefly as a laboratory oddity—an interesting if somewhat artificial tool for studies of the fundamental biology of viral infections But with the discovery of an ever-growing number of pathogenic and seemingly nonpathogenic viruses, the question of what might happen when a human being is exposed to mixed infection is perhaps not of purely academic interest but may prove to be of clinical importance as well This is suggested not only because the chance of acquiring concurrent infections grows with the number of possible risks but particularly in view of the mounting evidence indicating that viruses may persist and multiply in host cells for long periods of time without persistent pathologic consequences. A striking example of the latter phenomenon in man is the prolonged association of adenoviruses with adenoids and tonsils which must be inferred from the frequency with which they can be activated in cultures of these organs (Chap 29). It is almost certain that application of tissue culture methods will uncover similar relationships for other viruses and other organs.

There is as yet no direct proof for the hypothesis that such persistent infections of human cells may affect susceptibility to superinfecting viruses But under experimental conditions it has been possible to show, in a number of instances already mentioned, that carrier lines of cells are resistant to various active viruses This aspect offers a broad field for further exploration of immediate medical importance.

Apart from the possible protective role of a chronic carrier state, it seems unlikely that conditions favoring the establishment of effective interference would be often fulfilled in natural infection of man As has been discussed above, these conditions are highly specialized with regard to selection of suitable viruses, factors of dosage and timing, opportunity to infect the same cells concurrently. In order to interfere, two viruses would have to compete with each other either at the portal of entry or elsewhere in association with cells which both are able to infect Moreover, for interference to be effective as a protective mechanism, the entire or nearly the entire appropriate cell population would have to be involved. Therefore, it is small wonder that several pairs of viruses which are known to be capable of interfering in experimental hosts have been isolated together from individual patients. An example is the presence of Coxsackie and polioviruses in single stool specimens and concomitant demonstration of a simultaneous rise in antibody titers against both viruses in the patient's serum Interference between Coxsackie and polioviruses has been shown to occur in mice

That interference can exist in man under suitable conditions is illustrated by recent experimental work with dengue virus Simultaneous infection of human volunteers with attenuated yellow fever and active dengue viruses was followed by an attack of dengue much milder than in control patients infected with dengue virus alone. Specific cross immunity was not involved here, for yellow fever vaccine failed to protect against dengue virus given later It has also been found that the immunogenic effectiveness of two attenuated dengue strains and yellow fever vaccine is reduced when they are inoculated in the form of mixtures; depending on quantitative factors, antibody is produced against one to the exclusion of the other antigens Since the antigenic potency of these attenuated viruses seems to depend on their ability to multiply, it is likely that the failure to elicit a simultaneous immune response to each component is a result of interference (Schlesinger et al, 1956). This finding has obvious practical implications in view of the growing tendency to recommend a variety of attenuated viruses for immunization of man It should be noted

that *no* such mutual interference has been observed between inactivated viruses injected in the form of mixtures.

A further point of practical significance is the possible role of interference in vectors or intermediate hosts of human pathogens. For example, dengue and yellow fever viruses have been found to interfere in their common vector, *Aedes aegypti*, and interference between various arthropod-borne viruses belonging to Group A or Group B (Chap 12) has been demonstrated on numerous occasions in experimental animals or tissue culture It is conceivable that the geographic spread of these and other arthropod-borne viruses may be limited by such a mechanism, especially in view of the fact that insect vectors, once infected, remain so for the rest of their life.

REFERENCES

For references dated prior to 1930 see review by Henle, W (1950) and 2nd edition of this book, *Chapter 8*

Baluda, M A, 1957, Homologous interference by ultraviolet-inactivated Newcastle Disease virus, Virology *4*, 72-96

Cieciura, S J, Marcus, P I, and Puck, T T, 1957, The use of X-irradiated HeLa cell giants to detect latent virus in mammalian cells, Virology *3*, 426-427

Fazekas de St Groth, S, Isaacs, A, and Edney, M, 1952, Multiplication of influenza virus under conditions of interference, Nature, London *170*, 573

Gotlieb, T., and Hirst, G K, 1956, The experimental production of combination forms of virus VI Reactivation of influenza virus after inactivation by ultraviolet light Virology *2*, 235-248

Henle, W, 1950, Interference phenomena between animal viruses a review, J Immunol *64*, 203-236

Horsfall, F L, Jr, 1954, On the reproduction of influenza virus quantitative studies with procedures which enumerate infective and hemagglutinating virus particles, *J Exper Med* *100*, 135-161

Isaacs, A, 1957, Particle counts and infectivity titrations for animal viruses, *in* Advances in Virus Research, vol 4, pp 111-158, New York, Acad Press

Isaacs, A, and Lindenmann, J, 1957, Virus interference I. The interferon Proc Roy. Soc., London, s B, *147*, 258-267

Isaacs, A, Lindenmann, J, and Valentine, R C, 1957, Virus interference II Some properties of interferon Proc Royal Soc., London, s B, *147*, 268-273

Levine, S, 1958, Dynamics of heterologous interference between stable viruses in chick embryo fibroblast monolayers, Virology *5*, 150-167

von Magnus, P, 1951, Propagation of the PR8 strain of influenza A virus in chick embryos II The formation of "incomplete" virus following inoculation of large doses of seed virus Acta path et microbiol scandinav *28*, 278-293

Paucker, K, 1958, Personal communication from W Henle

Paucker, K, and Henle, W., 1955, Studies on host-virus interactions in the chick embryo-influenza virus system XII Further analyses of yields derived from heat-inactivated standard seeds J Exper Med *101*, 493-506

———, 1955, Studies on host virus interaction in the chick embryo-influenza virus system XI The effect of partial inactivation of standard seed virus at 37° C upon the progeny J Exper Med *101*, 479-492

Powell, W. F., and Pollard, E C, 1956, The effect of ionizing radiation on the interfering property of influenza virus, Virology *2*, 321-336

Powell, W F., and Setlow, R B, 1956, The effect of monochromatic UV radiation on the interfering property of influenza virus, Virology *2*, 337-343

Schlesinger, R W., 1950, Incomplete growth cycle of influenza virus in mouse brain, Proc Soc Exper Biol & Med *74*, 541-548

———, 1953, The relation of functionally deficient forms of influenza virus to viral developmnet, Cold Spring Harbor Symp Quant Biol *18*, 55-59

Schlesinger, [redacted] W, Gordon, I, Frankel, J W, Winter, J W, Patterson, P R, and Dorrance, W R, 1956, Clinical and serologic response of man to immunization with attenuated dengue and yellow fever viruses, J Immunol *77*, 352-364

Tyrrell, D A J, and Tamm, I, 1955, Prevention of virus interference by 2,5-dimethylbenzimidazole J Immunol *75*, 43-49

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6

Chemotherapy and Virus Infection

INTRODUCTION

At the present time no specific or effective therapy is available for virus diseases. Certain virus diseases can be prevented by immunization with infective or inactivated virus vaccines or by administration of specific antibodies. Moreover, in some infections administration of specific antibodies during the incubation period results in a milder form of disease. These preventive or modifying effects cannot be accomplished with any known drug. In contrast, numerous diseases caused by Chlamydozoaceae, rickettsiae, or bacteria can be effectively prevented or treated with appropriate drugs.

It is a matter of considerable theoretical interest as well as practical importance that highly effective antimicrobial compounds such as the tetracyclines fail to exert a beneficial effect in virus diseases except when secondary bacterial infection caused by drug-susceptible micro-organisms is present. Since not only viruses but also the Chlamydozoaceae and the rickettsiae are obligate intracellular parasites, the insusceptibility of viruses to present-day antimicrobial drugs cannot be due to this important biologic circumstance per se. The decisive difference between viruses on the one hand and Chlamydozoaceae and rickettsiae on the other presumably concerns the metabolic requirements and the intracellular conditions of multiplication of viruses in contrast with the larger agents. It is possible that the susceptibility of the larger agents derives from possession of some metabolic mechanism which the viruses, being smaller and simpler, lack. Possession of this hypothetical mechanism might endow the larger agents with a measure of independence from host cell metabolic activities and at the same time make them more vulnerable to selective inhibition.

Experimental studies in the laboratory involving thousands of chemical compounds and biologic materials have not yielded substances of sufficient promise to warrant a prediction that they might be useful in the treatment of human virus infections in the field. In fact, there is uncertainty as to whether potent and selective inhibitors of virus multiplication, if such were found, would prove to be useful in the treatment of virus diseases (Horsfall, 1955c, Tamm, 1956b). Thus, the validity of the classic principle of antimicrobial chemotherapy is in doubt so far as virus diseases are concerned.

Accordingly, there is undertaken in this chapter an analysis of the biologic and biochemical factors which determine the feasibility of various approaches, and the more meaningful results of experimental studies are presented within the framework of the biology and the biochemistry of virus infections. Because of lack of quantitative information on the relationship between virus multiplication and the development of lesions in man, and ignorance of the mechanism of viral damage, the discussion necessarily involves inferences and assumptions as well as facts.

PRINCIPLE OF ANTIMICROBIAL CHEMOTHERAPY

The fundamental principle of chemotherapy of infectious diseases caused by micro-organisms such as Chlamydozoaceae, rickettsiae, or bacteria may be stated as follows: Development of disease depends on an increase in the number of infecting pathogenic micro-organisms, if the number of micro-organisms is restricted through selective inhibition of multiplication or by selective killing of micro-organisms, disease fails to develop or is altered in favor of the host. It is implied that disease manifestations appear before the number of micro-organisms has reached maximal levels. Thus, in treatable microbial infections, symptoms and signs provide an early enough signal for initiation of chemotherapy based on the principle of restriction of the number of micro-organisms. No mention has been made of features which characterize individual microbial diseases, although it is recognized that large and important differences exist in regard to dissemination and maintenance of micro-organisms and lesions, mechanism of tissue damage, and other features. However these considerations do not affect the validity of the principle of antimicrobial chemotherapy, as defined above.

The therapeutically effective agents lack significant toxicity for the cells of the host at concentrations which are deleterious to the micro-organisms. The precise biochemical mechanism of the remarkable selective action of chemotherapeutic agents is not known.

APPLICABILITY OF THE PRINCIPLE OF ANTIMICROBIAL CHEMOTHERAPY TO VIRUS DISEASES

Whether or not the principle of antimicrobial chemotherapy is applicable to virus diseases depends on the degree of similarity between the biologic characteristics of virus diseases and the characteristics of treatable infectious diseases. This question is not concerned with the feasibility of development of highly selective virostatic or virocidal compounds but rather with the problem of whether such compounds, if found, would be useful in the treatment of virus diseases of man. The most important pertinent disease characteristic is the time relationship between multiplication of the infective agent and disease manifestations in virus diseases (Horsfall, 1955d). The information available on this point indicates that the classic principle of chemotherapy may be applicable in some but not in all virus diseases.

Interval Between Infection and Disease Manifestations

General Considerations. The feasibility of chemotherapeutic intervention directed against the infecting virus depends on the length of the interval between the first manifestations of disease and completion of those reactions which trigger the development of pathologic changes leading to the full-blown disease picture. This interval may be shorter than that between the first manifestations of disease and the time when maximal virus concentration is reached. Production of new virus particles proceeds in two steps: (1) synthesis of virus materials, and (2) assembly of new particles. It is possible that the process of synthesis of virus materials or the newly synthesized virus materials themselves can damage the host cell. In either case the infected cells may be on their way to degeneration and death before significant numbers of new virus particles have appeared in the cell. Results which have been obtained in studies on chemical inhibition of bacteriophage reproduction are of considerable interest in this connection. Chloramphenicol inhibits protein synthesis, and if added early in the latent period, it prevents both virus reproduction and host cell lysis (Bozeman et al., 1954). However, the concentration of chloramphenicol required to inhibit bacteriophage reproduction is closely similar to that which inhibits bacterial multiplication, and attempts to recover viable bacteria through drug treatment of infected bacterial cells have been unsuccessful. Proflavine interferes mainly with the final assembly of mature virus particles from precursor materials and not with the synthesis of new phage nucleic acid or protein (Foster, 1948). Although no new infective phage particles are formed in the presence of proflavine, lysis of treated bacterial cells occurs at the same time as that of untreated control cells.

The degeneration of a relatively small number of individual cells widely scattered in an

organ does not necessarily lead to manifestations of disease It is likely that a certain minimal local concentration of degenerating cells is necessary to give rise to symptoms or signs of disease Some disease manifestations clearly reflect loss of function of parenchymal cells, whereas others reflect secondary inflammatory changes in the tissues.

During the incubation period virus multiplication takes place probably both at sites of initial infection and at daughter sites It is to be expected that with different viruses infecting different tissues the time from infection to the appearance of disease manifestations would be variable because there could be differences in the rate of reproductive processes, in the rate and the manner of spread of infection, in the time when the chain of events is initiated leading to cytopathologic changes, in the rate of development of cytopathologic changes, and in the rate of development and the extent of inflammatory changes

In some diseases the virus may multiply first in one organ system, without causing detectable damage to the cells involved, and then in another, this time causing pathologic changes In such a case disease manifestations may be delayed until some time after infection of the second organ system, as in poliomyelitis (Chap 23).

Specific Diseases. In the absence of data regarding the concentration of virus materials and particles during the incubation period as well as during the various phases of disease, and regarding the precise pathogenesis and kinetics of viral lesions in man, the question as to the interval between the first manifestations of disease and those reactions which trigger the sequence of pathologic changes cannot be unequivocally answered for any of the virus infections afflicting man. Useful available information concerns the incubation period and the period of communicability (see Chapters on specific diseases and Control of Communicable Diseases in Man, 1955). These periods are known in most recognized virus diseases, the latter with much less precision than the former. Moreover, interpretation of the period of communicability for the present purposes is difficult The onset of the period of communicability probably signifies that the infecting virus had multiplied to a considerable extent. In some virus diseases the period of communicability begins before the appearance of symptoms, persons infected with the virus of yellow fever, dengue, or phlebotomus fever become infective for the appropriate vector one day before the onset of clinical disease. Poliomyelitis virus is present in the nasopharynx and the feces for several days before onset of the disease, and the infected persons may communicate the disease.

The end of the period of communicability may signify a number of different things. It may reflect primarily the termination of multiplication of the infecting virus; however, the rate of disappearance of virus probably is also of considerable importance in determining the end of the period of communicability The rate of disappearance depends on a number of factors: development of neutralizing antibodies, thermal inactivation, pH effects, possible engulfment and inactivation by macrophages, excretion and others One or another factor may be of greatest importance in a given disease. It is noteworthy that in some virus diseases such as poliomyelitis and infectious hepatitis, acute signs and symptoms of disease disappear before excretion of virus in the stools stops This does not necessarily mean that virus continues to multiply in the brain or the liver beyond the acute phase of the disease; it is more likely that the carrier state is due to multiplication of the virus in cells other than the parenchymal cells of these organs

On the basis of the limited information available, few conclusions are warranted regarding the length of the interval between infection and onset of pathologic changes irrevocably leading to full-blown lesions, i e, the interval during which selective virostatic or virocidal agents might be employed effectively. However, it does appear likely that in virus diseases of fairly long duration in which progressive involvement occurs during the course of disease not all tissues which eventually become involved may be seeded with virus at the time of onset of signs and symptoms, or the seeding may be slight. Into this group of diseases may fall mumps and possibly poliomyelitis and other diseases Primary atypical pneumonia associated with the development of cold hemagglutinins and streptococcus MG agglutinins, and infectious mononucleosis, may also fall into this category, though the virus etiology of these diseases has not been established unequivocally Although infectious or serum hepatitis is frequently of long duration, early virus lesions tend to be widespread (Dible et al., 1943; Mallory, 1947).

Thus it appears that in at least some virus infections classic chemotherapeutic approaches aiming to restrict the number of virus particles (Horsfall, 1950) are based on correct premises It is obvious, however, that success will depend on early initiation of treatment

The classic chemotherapeutic approach has been shown to be valid in the treatment of PVM virus pneumonia in mice with the capsular polysaccharide from *K pneumoniae* (Horsfall and McCarty, 1947, Ginsberg and Horsfall, 1951) When the substance is given after considerable virus multiplication has taken place and definite pneumonia has developed, further multiplication of the virus is inhibited, and the progress of the pneumonia is retarded The mice survive, and the lesions ultimately resolve

However, in considering approaches to the chemotherapy of virus diseases other avenues should also be explored, for a number of reasons (1) In certain virus diseases the duration of the whole illness is so short that classic intervention may not be practicable, and in others shortness of the interval between onset of disease manifestations and maximal involvement of tissues may preclude the effective use of virostatic or virocidal agents (2) It is frequently difficult to make a specific diagnosis very early during the course of an illness Should broad-spectrum antiviral drugs become available, this fact may not significantly decrease their usefulness However, the use of drugs with a limited antiviral spectrum in relatively mild self-limiting diseases might be impractical unless helpful epidemiologic information is available In serious diseases therapeutic trial with combinations of drugs may be justified (3) Available evidence indicates that multiplication of viral agents is so closely linked to the metabolism of host cells that it may prove to be difficult indeed to discover or develop highly selective inhibitors of virus multiplication It may be even more difficult to find ways of inactivating virus particles, particularly within host cells, without damage to cells

Ways to Implement the Classic Principle

Much work has been done both on inhibition of virus multiplication and on inactivation of virus particles However, although numerous chemical compounds and biologic substances have been reported to possess virus inhibitory activity, relatively little work has been done on the mechanism of action of such compounds, and only a few serious attempts have been made to improve the characteristics of inhibitory compounds through systematic development of new derivatives (Tamm, 1956b; 1958) In most in vitro studies the toxicity aspect has been neglected The more meaningful results which have been obtained are discussed below

Since no highly selective virostatic or virocidal agents are available at present, and since it is impossible to predict when such agents might be discovered, renewed consideration should be given to the possibility that specific antibodies given in very large doses may cause a favorable alteration in the course of some of the more serious virus diseases of man Before the advent of antimicrobial chemotherapy it was shown that certain bacterial infections, e g , pneumonococcal pneumonia (Horsfall et al , 1937), could be treated effectively with specific antibodies Admittedly, numerous reports have appeared on the failure of specific antiserum to alter the course of disease in man or in experimental animals Furthermore, the biologic features of virus infections of man suggest that even very large doses of intravenously or topically applied antibodies may possibly be effective only in a very limited number of virus infections, and only if given early in the course of illness However, a restudy of the problem, in poliomyelitis, for example, seems to be indicated, since methods are now available for obtaining gamma globulin from human beings hyperimmunized against the 3 types of poliomyelitis virus, and since early treatment should be possible in a considerable proportion of cases of this disease

Several other ways of obtaining virostatic or virocidal effects, such as with the properdin system (Ginsberg and Wedgwood, 1956), with interfering viruses or interferon (Chap 5), merit further exploration. A better understanding of factors which limit the yield of virus per cell and of those which limit the spread of virus in natural infection might open up new possibilities along the line of the classic approach

Francis (1957) has recently re-emphasized

the importance of identification of cellular components with which viruses presumably interact, in the belief that it may prove to be possible to prepare protective inhibitory analogs of such components

APPROACHES OTHER THAN THE CLASSIC

Little work has been done and few promising experimental results have been reported regarding ways to counteract the effects of viruses on cells. This is not surprising, since understanding of the processes involved in virus-host interaction is extremely limited A statement of some of the factors involved might lead to much-needed experiments in this field. Approaching the problem from the side of the virus, it would be of great interest to know whether viral damage is due to the process of virus reproduction or its products. If the first be true, the conceivable mechanisms include depletion of nutrient materials and diversion of enzyme systems, in either case the resulting reduction in the amounts of metabolites available to the host cell might be damaging per se, or it might be harmful because of an associated imbalance in the metabolic equilibrium If on the other hand virus materials are toxic (Henle and Henle, 1946a, 1946b) or cytopathogenic (Ginsberg, 1951) in themselves, then it follows that the chain of events leading to cellular degeneration could be initiated by the infecting particles, by materials released from them at the time of infection, by newly synthesized virus materials or by newly assembled virus particles, the mechanism of action might concern interruption of vital enzyme reactions in cells, disturbances in the normal cellular regulatory mechanism which maintain metabolic equilibrium, or activation of host-cell enzymes such as the nucleases and the proteinases. It should be emphasized that primary viral effects may lead to multiple secondary reactions in host cells Indeed, it is probable that in a number of virus infections the infecting virus triggers a reaction pattern of injury which may be characteristic of the particular cell type involved or may be common to different cell types.

Rivers (1928) pointed out 30 years ago that the primary pathologic changes in all virus diseases are hyperplasia, hyperplasia followed by necrosis, or necrosis. In recent years numerous reports of stimulation of various cellular metabolic activities during the early phases of virus infection have appeared (Tamm, 1958). Stimulation has been shown to be followed by depression in a number of cases (Rafelson et al, 1949; Overman and Tamm, 1957). It appears possible that at least in certain virus infections stimulation represents a metabolic disturbance which causally leads to depression of metabolic activities and degenerative changes in cell structure.

Approaching the problem of virus-host interaction from the side of the cell, it would be of great interest to know the mechanism of host resistance. Viruses may fail to infect cells or they may infect and multiply but fail to cause pathologic changes It is not known whether these phenomena are due to negative or positive factors· whether resistant cells lack constituents which the viruses require for infection or cytopathogenicity, or whether they possess inhibitory components which render the viruses inactive

There is evidence that a proportion of damaged cells in the brain and the cord of monkeys infected with poliomyelitis virus may recover (Bodian, 1949) In such nerve cells changes are arrested in the stage of cytoplasmic chromatolysis, following which complete morphologic recovery of the cell may occur over a period of about a month or less, depending on the severity of injury It is impossible to be certain whether these cells were actually infected with poliomyelitis virus or whether they showed changes which were not directly related to the presence or the multiplication of virus However, in either case, an understanding of the mechanism of recovery might open up new avenues of approach to the problem of chemotherapy of virus diseases. Also, it would be of great interest to know whether recovery of cells takes place in other virus infections. This information is not available at present. On the other hand, it is well known that in certain organs, such as the liver and the respiratory tract, repair may take place through regeneration of parenchymal cells Recently, it has been emphasized (Horsfall and Tamm, 1957; Francis, 1957) that detailed consideration of recovery phenomena in virus diseases may lead

to fruitful experiments on how to support and restore infected cells

The role of the secondary inflammatory response during both the acute and the recovery phases of virus infections has not been defined as yet It is possible that at least in certain virus diseases infiltration of leukocytes and vascular reactions may serve to limit the infection; on the other hand, inflammatory changes might cause damage in uninfected parenchymal cells or aggravate lesions in infected ones If leukocytic infiltrations and vascular changes are not useful features of the host response in certain virus infections, means might be sought to suppress such changes

EXPERIMENTAL STUDIES

Certain chemical compounds and biologic materials are sufficiently active to protect animals against death from virus infection under restricted experimental conditions (Tamm, 1956b) However, it is unlikely that any of the agents which have shown some virus inhibitory or protective activity in experimental animals would prove to be useful in the therapy or even the prophylaxis of natural virus diseases in man or animals The reasons for this are several, depending on the compound and on the virus infection In most cases the amount of compound required to produce a significant reduction in mortality has been close to the toxic dose, and treatment has had to be initiated within a short time after virus inoculation, that is, before the onset of signs of disease With few exceptions it has been possible to demonstrate protection only with relatively small virus inocula, and only in a proportion of infected animals In a number of instances the compounds employed have been effective only when given by the same route as the virus, and frequently the amounts required have been large It has often been found that treated survivors are susceptible to reinfection with the same virus

Only a small number of compounds used with some success in animals have been studied for their precise mechanism of action on the cellular level It is also true that the mechanism of action of many compounds which have been used to inhibit viruses in tissue culture is not known However, it may be said that, without exception, virus inhibitory compounds have been found to have inhibitory effects on some metabolic activity of host cells wherever such effects have been looked for (Tamm, 1958).

Studies with influenza virus have provided an indication of what can be learned about metabolic aspects of virus multiplication by the use of inhibitory compounds The most telling findings may be summarized as follows Inhibitors of biosynthetic processes have made it possible to define steps in the viral reproductive sequence. It has been established with the aid of the β-linked D-ribofuranoside of 5,6-dichlorobenzimidazole (DRB) that synthesis of ribonucleic acid (RNA) precedes the emergence of soluble complement-fixing antigen or influenza-virus particles (Tamm and Tyrrell, 1954, Tamm, 1957) Results of studies with methoxinine (Ackermann and Francis, 1954, Ackermann and Maassab, 1954) indicate that synthesis of proteins also precedes the appearance of new virus particles However, it appears that the processes concerned with influenza virus multiplication which are inhibitable by methoxinine are of longer duration than those inhibitable by the riboturanoside of dichlorobenzimidazole

Results of studies with inhibitors of energy-yielding mechanisms have imparted much meaning to the finding that influenza virus multiplication requires oxygen (Ackermann, 1951b, Tamm, 1956a) The essential function of the Krebs cycle in virus multiplication was demonstrated with the aid of malonate and fluoroacetate (Ackermann, 1951b, c), and dinitrophenol was instrumental in the demonstration of dependence of virus multiplication on availability of adenosine triphosphate (Eaton, 1952, Ackermann and Johnson, 1953, Ingraham et al, 1953)

It is possible that oxygen may be required for biosynthetic processes which take place during the latent period but not for later assembly of particles, since pentamidine (Eaton et al, 1952) and 2,5-dimethylbenzimidazole (Tamm et al, 1953, Tamm, 1956a), at concentrations inhibitory to the oxygen uptake of host tissue, reduced the yield of virus when given during the early phases of multiplication but had no effect on yield after rapid increase in new virus had begun

These results constitute a mere beginning in the understanding of the metabolic requirements of influenza virus multiplying in the chorio-allantoic membrane from embryonated

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acid or protein synthesis might have a greater effect with more significant consequences on the synthesis of virus than host-cell material

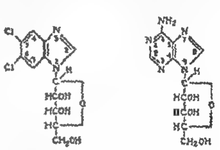
However, Horsfall (1950) cautioned earlier that, if the rate of turnover of cellular proteins and nucleic acids must be generally reduced to inhibit viral synthesis, it becomes highly improbable that attempts to discover effective chemotherapeutic agents active against viral infections will be fruitful, because substances which diminish either protein or nucleic acid synthesis within the cell would be directed against what are in all probability some of the most vital systems in cell biochemistry and could be expected to exert damaging effects on the host Indeed, if it proves to be true that no significant quantitative differences exist rendering the process of virus synthesis relatively more susceptible to the effects of some potent inhibitor, presently feasible studies on inhibition of nucleic acid or protein synthesis may not lead to the discovery of highly selective inhibitors of virus multiplication In any event, it is essential to proceed slowly and with caution and to explore the potentialities of new compounds widely in experimental animals before attempting to treat patients with them (Horsfall, 1954)

The experimental work reviewed below has been arranged according to mechanism of action of inhibitory compounds However, it should be emphasized, that very little direct and conclusive evidence is available deriving from studies under conditions of virus inhibition experiments

Experiments Aimed at Inhibition of Nucleic Acid Synthesis

Influenza and Mumps Viruses. Extensive investigations on the relationship between chemical structure of benzimidazole derivatives and their virus inhibitory activity have led to the synthesis of new compounds of very high activity (Tamm, 1956b, 1958) Less than 1 μg/ml of the β-D-ribofuranoside of monobromo-dichlorobenzimidazole causes marked inhibition of influenza B virus multiplication in the chorio-allantoic membrane from the embryonated chicken egg in vitro (Tamm, Folkers and Shunk, 1956), this compound is the most active inhibitor of virus multiplication reported

The structure of the β-D-ribofuranoside of dichlorobenzimidazole (DRB) (Tamm et al., 1954), a congener of the compound referred to above, and the time of its action early in the latent period of the reproductive cycle (Tamm and Tyrrell, 1954) suggested that β-linked ribofuranosides of halogenated benzimidazoles act through inhibition of RNA synthesis (Tamm, 1956b) This conclusion was borne out in experiments on inhibition by DRB of incorporation of adenosine-8-C^{14} into RNA of the host tissue (Tamm, 1957) and of orotic acid-6-C^{14} into RNA of isolated calf thymus nuclei (Allfrey et al, 1957) The diagram depicts the structural relationship between 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) and 9-β-D-ribofuranosyladenine (adenosine) The latter



5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (Inhibitor) | 9-β-D-Ribofuranosyladenine (In RNA)

Comparison of the structures of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) and 9-β-D-ribofuranosyladenine (adenosine)

is a natural metabolite present in RNA, adenosine mono-, di- and triphosphate and in several coenzymes Adenosine is capable of blocking the inhibitory action of DRB if low concentrations of the inhibitor are employed (Tamm, 1957) The combined evidence suggests that the β-linked ribofuranosides of halogenated benzimidazoles act as highly effective metabolic antagonists of a natural purine riboside

High inhibitory activity against the RNA-containing (Ada and Perry, 1954) influenza virus depends on two structural features: (1) the presence of the D-ribofuranose moiety in β-linkage at N1 in the imidazole ring, and (2)

chicken eggs in vitro Less is known about the requirements of other viruses.

No attempt will be made to review in detail the results of the numerous studies which bear on the problem of chemotherapy of virus infections Numerous recent reviews of work in this field are available (Horsfall, 1950, 1954, 1955a, b, c; Matthews and Smith, 1955, Hurst and Hull, 1956; Tamm, 1956b, 1958; Horsfall and Tamm, 1957, Francis, 1957). Instead, the more significant findings are discussed in terms of the varied mechanisms of action of the compounds and the substances used Work dealing with inhibition of viral multiplication or direct inactivation of the infective property of virus particles is discussed first. In later sections results bearing on other feasible approaches are summarized

Compounds and substances were chosen for review on a number of bases (1) because they provided examples illustrating certain modes of action, (2) because the accumulated evidence indicates that structural modification of presently available compounds might yield more effective compounds, (3) because protection against death was demonstrated in animal experiments, (4) because a synergistic effect was demonstrated, or (5) because the compounds used were highly active on a weight basis An attempt has been made to illustrate principles and concepts with the most complete examples available.

SELECTIVE INHIBITION OF VIRUS MULTIPLICATION

Present evidence indicates that the process of virus reproduction is dependent on host cells not only for building blocks such as nucleotides and amino acids but also for energy, and probably even for enzyme systems which may be required in the synthesis of virus materials. The intimacy of the host-virus relationship is strikingly reflected in the fact that synthesis of some virus materials appears to take place in the temporary absence of a limiting virus membrane (Morgan et al, 1954a, b; Hershey and Melechen, 1957). In view of these circumstances it is not surprising that highly selective inhibitors of virus multiplication have not yet been discovered or that their development is proving to be difficult.

A search for ways to inhibit synthesis of virus materials without inhibiting synthesis of corresponding host materials leads to the consideration of the chemical basis of the specificity of viruses. At the present time little information is available about the chemical basis of the heritable biologic specificity exhibited by viruses beyond the knowledge that nucleic acids and possibly proteins are involved. The relatively small number of different building blocks in virus nucleic acids and proteins and their common occurrence in biologic materials from many sources suggest that the sequence of nucleotides in nucleic acids and of amino acids in proteins may form the chemical basis for the biologic specificity of these chain molecules The discovery that in the deoxyribonucleic acid (DNA) from bacteriophages T2, T4 and T6 hydroxymethylcytosine replaces cytosine or its 5-methyl derivative (Wyatt and Cohen, 1953) has not led thus far to the finding of this base in other viruses or to the discovery of other virus specific bases There are indications that with proteins the shape of the molecule may also be important in determining biologic specificity

If the sequence of the repeating units were known it might be possible to design inhibitors which would interfere with the process of assembly of virus materials without interfering with the synthesis of host materials of a similar kind Because of limitations in knowledge, it is impossible to analyze the practicability of such an approach or to plan its execution at the present time However, it should be strongly emphasized that the chemistry of biosynthesis of nucleic acids and of proteins is in its infancy and that much progress may be expected within the next decade Also, empirical discovery of new inhibitors of these biosynthetic processes may greatly facilitate study of the steps and the intermediates involved.

As an alternative approach it has been suggested (Tamm, 1956b, 1958) that it may be more profitable to attempt exploitation of probable quantitative differences in the biosynthetic demands of the virus vs the host cell If considerable quantitative difference existed with respect to the conditions and the requirements of virus vs host material synthesis, an inhibitor of some step in nucleic

of dichlorobenzimidazole, namely, the α-linked D-arabinopyranoside did show enhanced selectivity with poliomyelitis virus. Although both compounds inhibited RNA synthesis at concentrations inhibitory for the poliomyelitis virus, surprisingly the arabinopyranoside also had a marked effect on protein synthesis (Nemes and Tamm, 1958). Thus, a high degree of inhibition of protein synthesis is not necessarily associated with a marked cytotoxic effect, and, furthermore, cytotoxicity and virus inhibitory activity do not necessarily go hand in hand

The biochemical action of DRB as an inhibitor of RNA synthesis was correlated with the kinetic finding that the greater part of its effect on poliomyelitis virus multiplication took place early during the latent period In contrast, the arabinopyranoside caused marked inhibition of protein synthesis, and with it inhibition of virus multiplication was obtained throughout the latent period and even beyond

Further support for the tentative conclusion that inhibition of protein synthesis provided an approach to the selective inhibition of poliomyelitis virus multiplication which was preferable to inhibition of RNA synthesis was obtained in experiments with azaserine and p-fluorophenylalanine Azaserine, which may be considered an inhibitor of RNA synthesis (Buchanan et al, 1957; Goldthwait, 1957), was considerably less selective as an inhibitor of poliomyelitis virus multiplication than p-fluorophenylalanine, which is probably an inhibitor of protein synthesis (Halvorson and Spiegelman, 1952)

Brown (1952) was the first to show that unsubstituted benzimidazole inhibited poliomyelitis virus in tissue culture A mixture of adenine, guanine and uracil partially blocked the inhibitory effect of benzimidazole This compound caused only a slight reduction in mortality of mice infected with the Lansing strain of poliomyelitis type II virus (Brown et al, 1953). Lack of a more marked effect is not surprising, since benzimidazole is an inhibitor of low activity, and since it has been demonstrated that at concentrations inhibitory to the multiplication of the Mahoney strain of poliomyelitis type I virus it causes a reduction in the oxygen uptake of HeLa cells (Gifford et al, 1954).

Experiments Aimed at Inhibition of Protein Synthesis

There are numerous reports in the literature on the inhibitory effect of amino acid analogs on the multiplication of influenza and poliomyelitis viruses, and on blocking of inhibition by appropriate amino acids The amino acid analogs which have been used include, among others, DL-methoxinine (Ackermann, 1951a), DL-ethionine (Ackermann, 1951a, Brown and Ackermann, 1951), p-fluorophenylalanine (Ackermann et al., 1954), L-canavanine (Pilcher et al, 1955), and threo-β-phenylserine (Dickinson and Thompson, 1957)

Where the amino acid analogs were examined for possible protective effect in animals, no evidence of protection could be found Two factors seem to be pertinent (1) low virus inhibitory activity of amino acid analogs, and (2) easy blocking of inhibition by the appropriate amino acid

It is hoped that some entirely new ways of inhibiting protein synthesis will be discovered

Inhibition of Energy-Yielding Mechanisms

Sodium monofluoroacetate, an inhibitor of citric acid oxidation, caused some inhibition of influenza A virus multiplication in the mouse lung (Ackermann, 1951c; Mogabgab and Horsfall, 1952) In mice infected with poliomyelitis type II virus sodium monofluoroacetate caused some suppression of virus multiplication and delayed slightly the onset of illness (Ainslie, 1952) In monkeys infected with poliomyelitis type I virus this compound caused a reduction in the number of animals developing paralysis but had no effect on the length of the incubation period (Francis et al, 1954)

Influenza virus multiplication in the embryonated egg was inhibited by 2,4-dinitrophenol and by sodium azide (Hannoun, 1954) The first compound acted presumably through interference with oxidative phosphorylation and the second through interference with oxidative processes

It seems doubtful that inhibition of energy-yielding processes will provide an approach to highly selective inhibition of virus multiplication

the presence of multiple halogen substituents in the benzenoid ring (Tamm, 1956b).

The selectivity of action of DRB was assessed in experiments in which the effects of this compound on a number of metabolic processes of the host tissue were determined at virus inhibitory concentrations. In addition, the effects of DRB on morphologic features of the tissue and the ability of cells to proliferate were also studied. In the presence of DRB at a concentration causing 75 per cent reduction in the yield of virus, moderate reduction in the uptake of adenosine-8-C^{14} into RNA of the uninfected chorio-allantoic membrane was observed (Tamm, 1957) At this concentration incorporation of C^{14}-L-alanine into the protein fraction and oxygen uptake of the tissue were not affected, and by microscopic examination large areas of the membrane showed no evidence of damage (Tamm, 1957, 1956a) At double this concentration of DRB, oxygen uptake was still not affected, but cells of the membrane grew out from explants at a reduced rate in roller-tube cultures (Tamm et al, 1954) On removal of the compound, cells proliferated rapidly, and the monolayer of cells surrounding the explant reached the size which had been attained earlier by the control cultures At a concentration 5 times greater than the 75 per cent virus inhibitory concentration microscopic examination showed considerable evidence of damage At even higher concentrations, microscopic damage was marked, oxygen uptake was reduced, and the membranes appeared grossly abnormal

Results of experiments in intact animals support the conclusion suggested by the in vitro findings, namely, that DRB shows some selectivity in its inhibitory action on influenza virus multiplication DRB inhibits influenza B and mumps virus multiplication in the embryonated egg, and influenza B virus multiplication in the mouse lung under restricted experimental conditions (Tamm, 1954; Tamm et al, 1954). This compound is capable of prolonging slightly the survival time of infected mice when administration is started 2 hours after inoculation of virus (Tamm, 1956c). However, DRB is not selective enough to be used in sufficient amounts to cause inhibition for a period of more than a few days without damage to the host

A comparison of DRB with 2,5-dimethylbenzimidazole, a derivative which was studied earlier (Tamm et al., 1952), is illuminating (Tamm, 1956a). Not only is DRB much more active as an inhibitor of virus multiplication than the 2,5-dimethyl compound, but it is also more selective as determined in experiments on: (1) inhibition of oxygen uptake by membranes; (2) cytotoxicity in membranes; (3) inhibition of proliferation of host cells, and (4) toxicity for chicken embryos and mice.

Thus, virus inhibitory activity and toxicity of compounds may vary independently, certain modifications in the structure of the inhibitor molecule have resulted in a disproportionate increase in virus inhibitory activity relative to toxicity, i e, in more selective derivatives Instances have also been observed where alteration of the molecular structure reduced toxicity without affecting virus inhibitory activity.

It appears that possibilities for increasing further the virus inhibitory effectiveness of benzimidazole derivatives through modifications in structure have not yet been exhausted

Poliomyelitis Virus. The structure-activity relationships established with a series of glycosides of chlorinated benzimidazoles and poliomyelitis type II virus in monkey kidney cells in vitro (Tamm and Nemes, 1957) paralleled those determined with influenza B virus (Tamm, 1956b) This is not surprising, since the nucleic acid in poliomyelitis virus is also of the RNA type (Schwerdt and Schaffer, 1955) However, it is noteworthy that quantitatively poliomyelitis virus was less susceptible to inhibition by the β-linked ribofuranoside of 5,6-dichlorobenzimidazole (DRB) than influenza virus The explanation for this difference probably concerns intracellular conditions of synthesis of virus materials and may involve factors such as quantitative differences in the metabolic requirements of these viruses, differences in the location of sites of synthesis of virus materials, differences in the effects of these viruses on the metabolism of host cells, or other features characteristic of each virus.

At the higher concentration required to inhibit poliomyelitis virus DRB caused microscopic damage to host cells (Tamm and Nemes, 1957). Another, less active glycoside

the Mahoney strain of poliomyelitis type I virus were protected against death by intravenous injection of very large amounts of specific antibodies, even though the administration of antibodies was delayed 48 hours. In the treated groups the incidence of paralysis was almost as high as that in control groups, but its degree was much less severe, and it involved only the lower extremities.

General Remarks. Since specific antibodies provide an excellent method of direct inactivation of virus particles in the blood or tissue fluids, any compound or component would have to be superior to antibodies in its protective action to gain justified attention. Clearly, compounds capable of selectively inactivating virus precursor materials or particles inside host cells would be of great interest.

VARIOUS OTHER MECHANISMS

Interference with Adsorption, Penetration, or Release of Virus Particles. Pretreatment of embryonated eggs with *Vibrio cholerae* filtrate, containing the so-called receptor-destroying enzyme (RDE), reduced the susceptibility of chick embryos to influenza virus (Stone, 1948a). Similar results were obtained in the mouse (Stone, 1948b). It is probable that RDE removed from the cell surface attachment sites necessary for adsorption of virus particles to host cells. Ackermann and Maassab (1954) have presented evidence that α-amino-*p*-methoxyphenylmethanesulfonic acid interferes with the penetration and the release of influenza virus from host cells.

Modification of Tissue Lesions. Xerosin, a biologic product prepared from culture filtrates of *Achromobacter xerosis*, suppressed the development of pneumonia in mice infected with influenza or Newcastle disease virus (Groupé et al., 1952, 1953). It should be emphasized that xerosin is neither virostatic nor virocidal, its effects appear to be mediated through the mechanisms of host response to injury. Indeed, xerosin appears to be primarily an anti-inflammatory agent (Ginsberg, 1955).

Newcastle disease virus does not multiply in the mouse lung but causes marked pneumonia and kills mice when given in sufficient quantities (Ginsberg, 1951). Xerosin protected mice against death from Newcastle disease virus pneumonia but caused only a delay in death when mice were infected with influenza virus. It appears that this substance has little if any effect on the primary damaging action of influenza virus on the epithelial cells lining the bronchi and the bronchioles but that it is capable of inhibiting secondary reactions such as edema, hemorrhage and infiltration (Ginsberg, 1955).

Nutritional, Hormonal and Environmental Factors. Hurst and Hull (1956) have emphasized that an animal weak or sickly from any cause may not respond typically to a virus infection. Thus, modified infection in animals whose state of health has been impaired by administration of toxic doses of a chemical substance, deficient diet, abnormal environmental conditions or by other circumstances, should not be accepted as evidence of successful chemotherapy.

Increased or decreased susceptibility may follow a wide variety of procedures which have no direct relevance to current concepts of chemotherapy but may provide information of value from the viewpoint of development of new approaches.

The fairly numerous reports in this area have been summarized by Hurst and Hull (1956).

MECHANISM UNKNOWN

It is important to point out that empirical testing of synthetic compounds and biologic materials has yielded agents capable of preventing death of animals from virus infections under restricted experimental conditions. For example, among synthetic compounds thiosemicarbazones protect mice against vaccinia virus (Hamre et al., 1951, Thompson et al., 1953), and quinacrine protects against Eastern equine encephalomyelitis virus (Hurst et al., 1952). Among biologic materials MS-8450, a culture filtrate from *Penicillium stoloniferum* (Powell and Culbertson, 1953), and helenine, an acetone precipitate of an extract obtained from the culture pellicle of *Penicillium funiculosum* (Shope, 1953), have shown protective activity against poliomyelitis virus in the monkey (Cochran et al., 1954, Cochran and Francis, 1956).

Pretreatment of monkey testicular cells in vitro with MS-8450 prevented the cyto-

Biochemical Mechanism Unknown

As was indicated above, the capsular polysaccharide from *K. pneumoniae* which inhibits the multiplication of pneumonia virus of mice (PVM) also shows chemotherapeutic activity (Horsfall and McCarty, 1947, Ginsberg and Horsfall, 1951) In addition, the polysaccharide is a highly active inhibitor of mumps virus multiplication in the embryonated egg (Ginsberg et al., 1948).

Certain acridines (Eaton et al, 1951). and pentamidine and stilbamidine (Eaton et al., 1952) are highly active inhibitors of influenza and mumps virus multiplication in tissue culture At virus inhibitory concentrations these compounds inhibited outgrowth of fibroblasts from tissue explants, the acridines had no effect on oxygen uptake or glycolysis, whereas pentamidine inhibited respiration

General Remarks

It should be emphasized that in all of the studies referred to the compounds employed did not inactivate the infective property of virus particles. The effects observed probably were due to reduction in the rate of virus reproduction

As far as is known, viruses do not possess energy-yielding mechanisms of their own Therefore, interference with energy-yielding mechanisms reduces the yield of virus wholly through effects on host-cell metabolism Compounds currently employed to inhibit nucleic acid or protein synthesis probably interfere with the synthesis or the utilization of low-molecular intermediates or precursors common for host and virus materials It is likely that reactions on this level are catalyzed by host-cell enzymes Thus, it is possible that all present-day inhibitors of biosynthetic processes reduce the yield of virus through inhibition of host-cell processes.

DIRECT INACTIVATION OF VIRUS PARTICLES

Chemical Compounds. Embryonated eggs were protected against death from Newcastle disease virus infection by treatment with small amounts of β-ethoxy-α-ketobutyraldehyde hydrate (Kethoxal). This and related compounds possess virocidal activity against Newcastle disease and certain other viruses (McLimans et al., 1957). It is likely that protection of a lesser degree, demonstrated with polylysine, is also due to a direct effect on virus particles (Green et al, 1953).

It should be pointed out that no compounds have been reported to be capable of selectively inactivating virus particles or virus components inside host cells

Cell or Tissue Components. Specific inhibition of the lytic action of bacteriophage by bacterial extracts was first demonstrated by Levine and Frisch (1934). The active principle appeared to be carbohydrate in nature Subsequently, it was shown that the specific lipocarbohydrate of Phase II *Sh sonnei* inactivates T4 bacteriophage and causes release of the content of the viral membrane into the surrounding medium (Jesaitis and Goebel, 1955)

A mucopolysaccharide isolated from the intestinal tissue of the mouse specifically combines with the GDVII strain of Theiler's encephalomyelitis virus of mice (Mandel and Racker, 1953a, b). In the combined state the virus fails to infect host cells or to agglutinate red cells The reaction between the mucopolysaccharide and the virus requires the presence of electrolyte, and the complex can be readily dissociated by decreasing the electrolyte concentration (Mandel, 1957).

The specific mucoprotein substrate of influenza virus enzyme reduces the infectivity of influenza virus as tested by allantoic injection into embryonated eggs (Tamm and Horsfall, 1952). The conclusion suggests itself that in the combined state the virus is unable to infect allantoic cells Since the virus is capable of enzymatically freeing itself from combination with the mucoprotein, the effect of the mucoprotein on infectivity is probably indirect and presumably is due to thermal inactivation, in the allantoic cavity, of the infective property of temporarily combined virus It is of considerable interest that periodate-treated mucoprotein, while capable of combining with influenza virus, is insusceptible to its enzymatic action. This modified substrate also causes a reduction in the infectivity of influenza virus for embryonated eggs (Burnet, 1948).

Antibodies. Macnamara and Morgan (1932) reported that human immune serum in the amount of 50 ml given in part intrathecally and in part intravenously reduced the extent of paralysis and the mortality in man from poliomyelitis if administered during the preparalytic stage but not when it was given after onset of paralysis. Recently, Liu et al. (1957) found that rhesus monkeys infected intraspinally in the lumbar region with

the propagation of poliomyelitis virus in tissue culture, J Immunol 69, 441-450
Brown, G C., and Ackermann, W W, 1951, Effect of dl-ethionine on poliomyelitis virus growth in tissue culture, Proc Soc Exper Biol & Med 77, 367-369
Brown, G C, Craig, H E, and Kandel, A., 1953, Effect of benzimidazole on experimental poliomyelitis in mice and monkeys, Proc Soc Exper Biol & Med 83, 408-411
Burnet, F M, 1948, Mucins and mucoids in relation to influenza virus action IV Inhibition by purified mucoid of infection and hemagglutination with the virus strain WSE Australian J Exper Biol & M Sc 26, 381-387
Cochran, K W, Brown, G C, and Francis, T, Jr, 1954, Antiviral action of a mold filtrate on experimental poliomyelitis in cynomolgus monkeys Proc Soc Exper Biol & Med 85, 104-106
Cochran, K. W, and Francis, T, Jr, 1956, Antiviral action of helenine on experimental poliomyelitis, Proc Soc Exper Biol & Med 92, 230-232
Control of Communicable Diseases in Man, 1955, ed 8, New York, Am Pub Health Assoc
Dible, D J, McMichael, J, and Sherlock, S P V, 1943, Pathology of acute hepatitis Aspiration biopsy studies of epidemic, arsenotherapy and serum jaundice Lancet 2, 402-405
Dickinson, L, and Thompson, M, 1957, The antiviral action of threo-β phenylserine, Brit J Pharmacol 12, 66-73
Eaton, M D, 1952, Observations on growth of virus and the energy-yielding activities of the host cell, Arch ges Virusforsch 5, 53 77
Eaton, M D., Cheever, F S, and Levenson, C G, 1951, Further observations of the effect of acridines on the growth of viruses, J Immunol 66, 463-476
Eaton, M D, Perry, M E, Levenson, C G, and Gocke, I M, 1952, Studies on the mode of action of aromatic diamidines on influenza and mumps virus in tissue culture, J Immunol 68, 321-333
Foster, R A C, 1948, An analysis of the action of proflavine on bacteriophage growth, J Bact 56, 795-809
Francis, T, Jr, 1958, Viral inhibition in Poliomyelitis Papers and Discussions Presented at the Fourth Internat Poliomyelitis Conf, Philadelphia, Lippincott
Francis, T, Jr, Brown, G C, and Kandel, A, 1954, Effect of fluoroacetate upon poliomyelitis in monkeys, Proc Soc Exper Biol & Med 85, 83-85
Gifford, G E, Robertson, H E, and Syverton, J T, 1954, Application of manometric method to testing chemical agents in vitro for interference with poliomyelitis virus synthesis, Proc Soc Exper Biol & Med 86, 515-522
Ginsberg, H S, 1951, Mechanism of production of pulmonary lesions in mice by Newcastle disease virus (NDV), J Exper Med 94, 191-211
———, 1955, Suppression of influenza viral pneumonia in mice by the non-specific action of xerosin, J Immunol 75, 430-440
Ginsberg, H S, Goebel, W F, and Horsfall, F L, Jr, 1948, The inhibitory effect of polysaccharide on mumps virus multiplication, J Exper Med 87, 385-410
Ginsberg, H S, and Horsfall, F L, Jr, 1951, Therapy of infection with pneumonia virus of mice (PVM), effect of a polysaccharide on the multiplication cycles of the virus and on the course of the viral pneumonia J Exper Med 93, 161-171
Ginsberg, H S, and Wedgwood, R J, 1956, Inactivation of virus by the properdin system, Ann New York Acad Sc 66, 251-261
Green, M., Stahmann, M A, and Rasmussen, A F, 1953, Protection of embryonated eggs infected with infectious bronchitis or Newcastle disease virus by polypeptides, Proc Soc Exper Biol & Med 83, 641-643
Groupé, V, Pugh, L M, and Levine, A S, 1952, Suppression of viral pneumonia in mice by a microbial product (APM), Proc Soc Exper Biol & Med 80, 710-714
———, 1953, Mechanism of suppression of nontransmissible pneumonia in mice induced by Newcastle disease virus, Science 118, 187-191
Halvorson, H O, and Spiegelman, S, 1952, The inhibition of enzyme formation by amino acid analogues, J Bact 64, 207-221
Hamre, D, Brownlee, K A, and Donovick, R, 1951, Studies on the chemotherapy of vaccinia virus II The activity of some thiosemicarbazones J Immunol 67, 305-312
Hannoun, C, 1954, Effects du 2-4-dinitrophénol et du nitrite de sodium le système embryon de Poulet-virus grippal Compt rend Acad sc, Paris, 238, 1172-1174
Henle, G and Henle, W, 1946a, Studies on the toxicity of influenza viruses I The effect of intracerebral injection of influenza viruses J Exper Med 84, 623-637
Henle, W, and Henle, G, 1946b, Studies on the toxicity of influenza viruses II The effect of intra-abdominal and intravenous injection of influenza viruses J Exper Med 84, 639-660
Hershey, A D, and Melechen, N E, 1957, Synthesis of phage-precursor nucleic acid in the presence of chloramphenicol, Virology 3, 207-236
Horsfall, F L, Jr, 1950, Approaches to the control of viral diseases, Bact Rev 14, 219-224
———, 1954, Chemotherapy of respiratory viral diseases, Pediatrics 13, 593-598
———, 1955a, Approaches to the chemotherapy of viral diseases, Bull New York Acad Med 31, 783-793
———, 1955b, The inhibition of virus reproduction by chemical substances, in Perspectives and Horizons in Microbiology, Waksman, S A (ed), pp 152-167, New Brunswick, Rutgers
———, 1955c, Virus reproduction and virus disease, Canad M A J 73, 778-782
———, 1955d, On the consequences of virus reproduction, Tr A Am Physicians 68, 54-63
Horsfall, F L, Jr, Goodner, K, MacLeod, C M, and Harris, A H, 2nd, 1937, Antipneumococcus rabbit serum as a therapeutic agent in lobar pneumonia, J A M A 108, 1483-1490

pathogenic effect of poliomyelitis virus (Hull and Lavelle, 1953) It is not established whether this unique result was due to prevention of infection, inhibition of virus multiplication, alteration of host cell response, or to some other mechanism

Exploration of the mechanism of action of the various agents may lead to new insights into the biology and the biochemistry of virus infections

SYNERGISM

Combined treatment of mice infected with Columbia SK encephalomyelitis virus with helenine and specific immune serum afforded a considerably greater degree of protection against death than treatment with either agent alone (Shope, 1953). Similar results were obtained with levo-γ-(o-chlorobenzyl)-δ-oxo-γ-phenyl caproic acid (caprochlorone) and human gamma globulin in mice infected with influenza virus (Liu et al, 1957).

The combined protective effect of isatin thiosemicarbazone and 5-(2′ 4′-dichlorophenoxy)thiouracil against vaccinia infection in mice was much greater than the effect of either compound given separately (Bauer, 1955)

CONCLUSIONS

Many different approaches to the problem of chemotherapy of virus diseases appear to be feasible. Among the most promising, currently practicable lines of approach are the following (1) development of highly selective inhibitors of virus multiplication, (2) exploration of the efficacy of large amounts of specific homologous antibodies; (3) determination of the mechanism and the role of inflammatory changes in virus infections

In view of the inherent limitations of any single approach it appears advisable to combine, whenever feasible, several approaches; a relatively small effect obtainable with a single procedure may be magnified when several methods of treatment are applied together.

The importance of fundamental investigations in the biology and the biochemistry of virus infections should not require emphasis. In a number of areas not enough is known even to formulate effective working hypotheses Susceptibility or resistance of cells to infection, viral cytopathogenicity, the nature and the mechanism of degenerative changes in cells, limiting factors which determine amount of virus produced, and recovery processes are some of the aspects of virus-host interaction, which, if understood, might lead to new approaches to chemotherapy of virus infections.

REFERENCES

Ackermann, W W, 1951a, The role of L-methionine in virus propagation, J Exper Med 93, 337-343

———, 1951b, Concerning the relation of the Krebs cycle to virus propagation, J Biol Chem 189, 421-428

———, 1951c, The relation of the Krebs cycle to viral synthesis II The effect of sodium fluoroacetate on the propagation of influenza virus in mice J Exper Med 93, 635-642

Ackermann, W W, and Francis, T, Jr, 1954, Characteristics of viral development in isolated animal tissues in Advances in Virus Research, vol 2, pp 81-108, New York, Acad Press

Ackermann, W W, and Johnson, R B, 1953, Some energy relations in a host-virus system, J Exper Med 97, 315-322

Ackermann, W W, and Maassab, H F, 1954, Growth characteristics of influenza virus Biochemical differentiation of stages of development J Exper Med 100, 329-339

Ackermann, W W., Rabson, A, and Kurtz, H, 1954, Growth characteristics of poliomyelitic virus in HeLa cell cultures lack of parallelism in cellular injury and virus increase J Exper Med 100, 437-450

Ada, G L, and Perry, B T, 1954, The nucleic acid content of influenza virus, Australian J Exper Biol & M Sc 32, 453-468

Ainslie, J D, 1952, The growth curve of the Lansing strain of poliomyelitic virus in mice: the effect of sodium monofluoroacetate and methionine sulfoximine on the early phase of growth of the virus J Exper Med 95, 9-28

Allfrey, V G, Mirsky, A E, and Osawa, S, 1957, Protein synthesis in isolated cell nuclei, J Gen Physiol 40, 451-490

Bauer, D J, 1955, The antiviral and synergic actions of isatin thiosemicarbazone and certain phenoxypyrimidines in vaccinia infection in mice, Brit J Exper Path 36, 105-114

Bodian, D, 1949, Poliomyelitis Pathologic anatomy in Poliomyelitis Papers and Discussions Presented at First Internat Poliomyelitis Conf., pp 62 84, Philadelphia, Lippincott

Bozeman, F M, Wisseman, C L, Jr, Hopps, H E, and Danauskas, J X, 1954, Action of chloramphenicol on T-1 bacteriophage I. Inhibition of intracellular multiplication J Bact. 67, 530-536

Brown, G C., 1952, The influence of chemicals on

the propagation of poliomyelitis virus in tissue culture, J. Immunol 69, 441-450

Brown, G C., and Ackermann, W W., 1951, Effect of DL-ethionine on poliomyelitis virus growth in tissue culture, Proc Soc. Exper Biol & Med 77, 367-369.

Brown, G C., Craig, D E., and Kandel, A., 1953, Effect of benzimidazole on experimental poliomyelitis in mice and monkeys, Proc Soc Exper Biol & Med 83, 408-411

Burnet, F M., 1948, Mucins and mucoids in relation to influenza virus action IV Inhibition by purified mucoid of infection and hemagglutination with the virus strain WSE Australian J Exper Biol & M Sc 26, 381-387

Cochran, K W, Brown, G C, and Francis, T, Jr, 1954, Antiviral action of a mold filtrate on experimental poliomyelitis in cynomolgus monkeys Proc Soc Exper Biol. & Med 85, 104-106

Cochran, K W, and Francis, T, Jr, 1956, Antiviral action of helenine on experimental poliomyelitis, Proc Soc Exper Biol & Med 92, 230-232

Control of Communicable Diseases in Man, 1955, ed 8, New York, Am Pub Health Assoc

Dible, D J, McMichael, J, and Sherlock, S P V, 1943, Pathology of acute hepatitis Aspiration biopsy studies of epidemic, arsenotherapy and serum jaundice Lancet 2, 402-408

Dickinson, L., and Thompson, M, 1957, The antiviral action of threo-β-phenylserine, Brit J Pharmacol 12, 66-73

Eaton, M D., 1952, Observations on growth of virus and the energy-yielding activities of the host cell, Arch ges Virusforsch 5, 53 72

Eaton, M D., Cheever F S, and Levenson, C G, 1951, Further observations of the effect of acridines on the growth of viruses, J Immunol 66, 463-476

Eaton, M D, Perry, M E, Levenson, C G, and Gocke, I M, 1952, Studies on the mode of action of aromatic diamidines on influenza and mumps virus in tissue culture, J Immunol 68, 321-333

Foster, R A C., 1948, An analysis of the action of proflavine on bacteriophage growth, J Bact 56, 795-809

Francis, T, Jr, 1958, Viral inhibition in Poliomyelitis Papers and Discussions Presented at the Fourth Internat Poliomyelitis Conf, Philadelphia, Lippincott

Francis, T, Jr, Brown, G C, and Kandel, A, 1954, Effect of fluoroacetate upon poliomyelitis in monkeys, Proc Soc Exper Biol & Med 85, 83-85

Gifford, G E, Robertson, H E, and Syverton, J T, 1954, Application of manometric method in testing chemical agents in vitro for interference with poliomyelitis virus synthesis, Proc Soc Exper Biol. & Med 86, 515-522

Ginsberg, H S, 1951, Mechanism of production of pulmonary lesions in mice by Newcastle disease virus (NDV), J Exper Med 94, 191-211

———, 1955, Suppression of influenza viral pneumonia in mice by the non-specific action of zerosin, J Immunol 75, 430-440

Ginsberg, H S, Goebel, W F, and Horsfall, F L, Jr., 1948, The inhibitory effect of polysaccharide on mumps virus multiplication, J Exper Med 87, 385-410

Ginsberg, H S, and Horsfall, F L, Jr., 1951, Therapy of infection with pneumonia virus of mice (PVM), effect of a polysaccharide on the multiplication cycles of the virus and on the course of the viral pneumonia J Exper Med 93, 161-171

Ginsberg, H S, and Wedgwood, R J, 1956, Inactivation of virus by the properdin system, Ann New York Acad Sc 66, 251-261

Green, M., Stahmann, M A., and Rasmussen, A F, 1953, Protection of embryonated eggs infected with infectious bronchitis or Newcastle disease virus by polypeptides, Proc Soc Exper Biol & Med 83, 641-643

Groupé, V., Pugh, L M, and Levine, A S, 1952, Suppression of viral pneumonia in mice by a microbial product (APM), Proc Soc Exper Biol & Med 80, 710-714

———, 1953, Mechanism of suppression of non-transmissible pneumonia in mice induced by Newcastle disease virus, Science 118, 187-191

Halvorson, H O, and Spiegelman, S, 1952, The inhibition of enzyme formation by amino acid analogues, J Bact 64, 207-221

Hamre, D, Brownlee, K A, and Donovick, R, 1951, Studies on the chemotherapy of vaccinia virus II The activity of some thiosemicarbazones J Immunol 67, 305-312

Hannoun, C, 1954, Effects du 2 4-dinitrophenol et du nitrure de sodium le système embryon de Poulet-virus grippal Compt rend Acad sc, Paris, 238, 1172-1174

Henle, G and Henle, W, 1946a, Studies on the toxicity of influenza viruses I The effect of intra cerebral injection of influenza viruses J Exper Med 84, 623-637

Henle, W, and Henle, G, 1946b, Studies on the toxicity of influenza viruses II The effect of intra-abdominal and intravenous injection of influenza viruses J Exper Med 84, 639-660

Hershey, A D., and Melechen, N E, 1957, Synthesis of phage-precursor nucleic acid in the presence of chloramphenicol, Virology 3, 207 236

Horsfall, F L., Jr, 1950, Approaches to the control of viral diseases, Bact Rev 14, 219-224

———, 1954, Chemotherapy of respiratory viral diseases, Pediatrics 13, 593-598

———, 1955a, Approaches to the chemotherapy of viral diseases, Bull New York Acad Med 31, 783-793

———, 1955b, The inhibition of virus reproduction by chemical substances, in Perspectives and Horizons in Microbiology, Waksman, S A, (ed), pp 152-162, New Brunswick, Rutgers

———, 1955c, Virus reproduction and virus disease, Canad M A J 73, 778-781

———, 1955d, On the consequences of virus reproduction, Tr A Am Physicians 68, 54-63

Horsfall, F L, Jr, Goodner, K, MacLeod, C M, and Harris, A H, 2nd, 1937, Antipneumococcus rabbit serum as a therapeutic agent in lobar pneumonia, J A M A 108, 1483-1490

Horsfall, F. L., Jr., and McCarty, M., *1947*, The modifying effects of certain substances of bacterial origin on the course of infection with pneumonia virus of mice (PVM), *J Exper Med. 85, 623-646*

Horsfall, F L., Jr., and Tamm, I, 1957, Chemotherapy of viral and rickettsial diseases, Ann Rev Microbiol *11*, 339-370

Hull, R N, and Lavelle, J M, 1953, Inhibition of cytopathogenic effect of poliomyelitis viruses in tissue culture by antibiotic M-8450, Proc Soc Exper Biol & Med *83*, 787-790

Hurst, E W, and Hull, R, 1956, The chemotherapy of virus diseases, with brief consideration of the influence of dietary, hormonal and other factors in virus infections, Pharmacol Rev *8*, 199-263

Hurst, E W, Melvin, P, and Peters, J M, 1952, The prevention of encephalitis due to the viruses of Eastern equine encephalomyelitis and louping-ill experiments with trypan red, mepacrine and many other substances Brit J Pharmacol *7*, 455-472

Ingraham, J L, Roby, T O, and Peterson, J. H, 1953, Effect of 2,4-dinitrophenol and sodium azide on host cell metabolism and influenza A virus, Arch Biochem *46*, *215-220*

Jesaitis, M A, and Goebel, W F, 1955, Lysis of T4 phage by the specific lipocarbohydrate of Phase II *Shigella sonnei*, J Exper Med *102*, *733-752*

Levine, P, and Frisch, A W, 1934, On specific inhibition of bacteriophage action by bacterial extracts, J Exper Med *59*, *213-228*.

Liu, O C, Carter, J E, DeSanctis, A N, Geating, J A, and Hampil, B, *1958*, *A study of the effect of antiserum on poliovirus infection induced by the intraspinal inoculation of rhesus monkeys*, J Immunol *80*, 106-113

Liu, O C, Carter, J E, Malsberger, R G, DeSanctis, A N, and Hampil, B, 1957, Studies on *the chemotherapy of viral infections II The effect of caprochlorone on influenza virus infections in mice J* Immunol *78*, *222-227*

Macnamara, J, and Morgan, F G, 1932, Poliomyelo-encephalitis in Victoria (1925-1931) Treatment by human immune serum Lancet *1*, 469-472, 527-532

Mallory, T B, *1947*, The pathology of epidemic hepatitis, J A M A *134*, *655-662*

Mandel, B, 1957, Inhibition of Theiler's encephalomyelitis virus (GDVII strain) of mice by an intestinal mucopolysaccharide III Studies on factors that influence the virus-inhibitor reaction Virology *3*, 444-463,

Mandel, B., and Racker, E, 1953a, Inhibition of Theiler's encephalomyelitis virus (GDVII strain) of mice by an intestinal mucopolysaccharide, I Biological properties and mechanism of action J. *Exper Med 98*, 399-415

———, 1953b, Inhibition of Theiler's encephalomyelitis virus (GDVII strain) of mice by an intestinal mucopolysaccharide. II Purification and properties of the mucopolysaccharide *J*. Exper. Med *98*, 417-426

Matthews, R E F, and Smith, J D., 1955, The chemotherapy of viruses *in* Advances in Virus Research, vol. *3*, pp. 49-148, New York, Acad Press

McLimans, W F., Underwood, G E, Slater, E A, Davis, E V, and Siem, R A, *1957*, Antiviral activity of dicarbonyls and related compounds in embryonated eggs J Immunol *78*, 104-111

Mogabgab, W. J, and Horsfall, F L, Jr, 1952, Effect of sodium monofluoroacetate on the multiplication of influenza viruses, mumps virus, and pneumonia virus of mice (PVM), J Exper Med *96*, 531-548

Morgan, C., Ellison, S. A, Rose, H M., and Moore, D H, 1954a, Structure and development of viruses as observed in the electron microscope I Herpes simplex virus J Exper Med *100*, 195-202

— [illegible]

N [illegible] biochemical aspects of poliovirus multiplication, VII International Congress for Microbiology. In press

Overman, J R, and Tamm, I, 1957, Multiplication of vaccinia virus in the chorioallantoic membrane *in vitro*, Virology *3*, 173-184

Pilcher, K S, Soike, K F, Smith, V H, Trosper, F, and Folston, B., 1955, Inhibition of multiplication of Lee influenza virus by canavanine, Proc. Soc Exper Biol & Med *88*, 79-86

Powell, H M, and Culbertson, C G, 1953, Action of an antiviral mold filtrate against MEF1 poliomyelitis virus in mice, Proc Soc Exper Biol & Med *83*, 161-163

Rafelson, M E, Jr, Winzler, R J, and Pearson, H E, 1949, The effects of Theiler's GDVII virus on P^{32} uptake by minced one day-old mouse brain, J Biol Chem *181*, 583-593

Rivers, T M, *1928*, Some *general aspects of pathological conditions caused by filterable viruses, Am* J Path *4*, 91-124

Schwerdt, C E, and Schaffer, F L, 1955, Some physical and chemical properties of purified poliomyelitis virus preparations, Ann New York Acad Sc *61*, 740-750

Shope, R. E, 1953, *An antiviral substance from Penicillium funiculosum* I Effect upon infection in mice with swine influenza virus and Columbia SK encephalomyelitis virus J Exper Med *97*, 601-625

Stone, J D, 1948a, *Prevention of virus infection with enzyme of V cholerae I Studies with viruses of mumps-influenza group in chick embryos* Australian J Exper Biol & M Sc *26*, 49-64

———, *1948b*, Prevention of virus infection with *enzyme of V. cholerae* II Studies with influenza virus in mice Australian J Exper Biol & M Sc *26*, 287-298

Tamm, I, 1954, Inhibition of influenza and mumps virus multiplication by 4,5,6-(or 5,6,7-)trichloro-1-β-D-ribofuranosylbenzimidazole, Science *120*, 847-848

———, 1956a, Selective *chemical inhibition of influenza B virus multiplication, J Bact. 72, 42-53*

Tamm, I., 1956b, Antiviral chemotherapy, Yale J Biol & Med 29, 33–49
——, 1956c, Unpublished data
——, 1957, Ribonucleic acid synthesis and influenza virus multiplication, Science 126, 1235–1236
——, 1955, Selective inhibition of virus multiplication, *in* "Strategy of Chemotherapy" Eighth Symposium of the Society for General Microbiology, Cambridge, Cambridge Univ Press
Tamm, I, Folkers, K., and Horsfall, F L, Jr, 1952, Inhibition of influenza virus multiplication by 2,5-dimethylbenzimidazole, Yale J Biol & Med 24, 559–567
——, 1953, Inhibition of influenza virus multiplication by alkyl derivatives of benzimidazole I Kinetic aspects of inhibition by 2,5-dimethylbenzimidazole as measured by infectivity titrations J Exper Med 98, 219–227
Tamm, I, Folkers, K, and Shunk, C H, 1956, High inhibitory activity of certain halogenated ribofuranosylbenzimidazoles on influenza B virus multiplication, J Bact 72, 54–58
Tamm, I, Folkers, K, Shunk, C H, and Horsfall, F L, Jr, 1954, Inhibition of influenza virus multiplication by N-glycosides of benzimidazoles, J Exper Med 99, 227–250
Tamm, I, and Horsfall, F L, Jr, 1952, A mucoprotein derived from human urine which reacts with influenza, mumps, and Newcastle disease viruses, J Exper Med 95, 71–97
Tamm, I, and Nemes, M M, 1957, Glycosides of chlorobenzimidazoles as inhibitors of poliovirus multiplication, Virology 4, 483–498
Tamm, I, and Tyrrell D A J, 1954, Influenza virus multiplication in the chorioallantoic membrane *in vitro* kinetic aspects of inhibition by 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole J Exper Med 100, 541–562
Thompson, R L, Minton, S A, Jr, Officer, J E, and Hitchings, G H, 1953, Effect of heterocyclic and other thiosemicarbazones on vaccinia infection in the mouse, J Immunol 70, 229–234
Wyatt, G R, and Cohen, S S, 1953, The bases of the nucleic acids of some bacterial and animal viruses The occurrence of 5-hydroxymethylcytosine, Biochem J 55, 774–782

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7

Bacteriophages

INTRODUCTION

Owing to the rapidly increasing scope of the subject in recent years, a chapter on bacteriophages can only sketch an outline of present knowledge Some historical notes will serve to add perspective

D'Herelle (1917, 1926) discovered bacteriophages and worked out the lytic cycle of their growth in general terms. attachment of phage particle to bacterium, penetration within, intracellular multiplication of phage, and cellular lysis liberating new phage particles He regarded the phages as members of a single species of parasitic ultramicrobe, but it is doubtful whether he should be given additional credit for this view, which was incorrect in important respects However, his main line of research is being pursued to this day in the direction he gave it

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Two developments, in fact, beginning about this same time, were to alter radically earlier notions about phages At the same time or even before, similar revolutions were taking place in the study of animal and plant viruses For phages these developments begin with the work of Doermann and Dissosway (1949, see Doermann, 1953) showing that phages multiply in noninfective form inside bacteria they are going to lyse, and with remarkably fruitful inquiries into the fundamental nature of lysogeny, by Lwoff and Gutmann (1950), Bertani (1951) and the Lederbergs (1953) The work with lysogenic bacteria also showed that phages could multiply in noninfective form and, moreover, could make lasting peace with their bacterial hosts Anyone interested in early history may read, besides the papers cited, general discussions by Luria (1950) and Hershey (1952), which happen to have been written at a critical time, and the comprehensive review by Lwoff (1953), written when some of the main issues were being settled

For the remainder of this chapter we ignore past history and focus directly on current research, in which the discerning reader will detect a bias. The nature of this bias should perhaps be stated at the outset

Phages are now known to contain a functional equivalent to the chromosomes of other organisms Rightly or wrongly, it is believed that a phage chromosome is a single molecule of deoxyribonucleic acid, structurally very different from the chromosomes of other organisms Therefore, one major aim of phage research is to relate viral inheritance and function to nucleic acid structure and function; to create, in a word, a science of molecular genetics The gains for medical biology from this pursuit are scarcely predictable.

Phages also play subtle and complex roles in bacterial inheritance and function Elucidation of this aspect of phage biology is revealing new modes of variation in the potentialities of cells and should help to define the place of viruses in cellular economy, pathologic and otherwise The importance of this goal to medical and biologic problems has been recognized for a long time

The bias mentioned above consists of conscious cleaving to the stated goals, which are ambitious ones. Present results suggest that failure to reach them will not prove to be unrewarding If so, the bias is harmless

Finally, it should be made clear that the phages represent a moderately homogeneous class of highly specialized viruses, adapted to cohabitation with bacteria For one thing, they all seem to contain deoxyribonucleic acid as a major component, which is true of few or no animal viruses It would be naive, to say the least, to look to the bacteriophages for direct clues to the pathogenesis of specific viral diseases of man

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The general character of bacteriophages is best conveyed by a brief description of the cycle of their growth Extracellular phage particles are inert objects often called *mature* or *resting* particles They are found wherever bacteria occur They first show signs of life when they encounter a specifically receptive host bacterium Following attachment to the cell, the phage particle literally splits into two parts of roughly equal mass, one composed chiefly of deoxyribonucleic acid (DNA) that penetrates inside the bacterium, the other composed chiefly of protein that does not

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7

Bacteriophages

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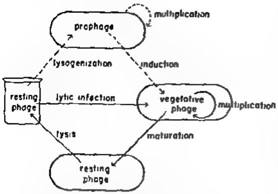

FIG 43. The life cycle of bacteriophages All stages are intrabacterial except the one represented in a beaker. The broken lines indicate processes forbidden to virulent phages (Hershey, A D, 1957, Bacteriophages as genetic and biochemical systems *in* Advances in Virus Research, *4*:25-61, New York, Acad Press)

penetrate This manner of penetration, often called *injection*, accounts for one of the main characteristics of the growth cycle, when mature phage particles are formed in an infected cell they are new particles, arising in a bacterium that previously contained nothing similar to them.

Infection of the appropriate host bacterium with a phage particle typically leads to the production of many offspring particles that can escape from the bacterium only when it lyses, which it commonly does some minutes after infection This sequence of events is called the *lytic cycle* of phage growth

The infection can proceed in a very different manner from that described above Usually rarely, but in some systems very frequently, the infected cell survives and multiplies to yield infected daughter cells. These infected cells do not often produce mature phage particles or lyse during continued growth However, an occasional daughter cell may do so, and the infection is recognized mainly by this tendency A pure culture of such viable infected cells is called a *lysogenic* culture, the infection giving rise to it is called *lysogenization*, and the occasional lapse of a lysogenic bacterium into the lytic cycle of phage growth is called *spontaneous phage production* or, especially when brought about by experimental means, *induction*. The lytic cycle following induction and the lytic cycle following primary infection are identical.

All naturally occurring phages can initiate the lytic cycle of growth, which is necessary for their perpetuation. Only some phages, called *temperate*, can lysogenize as well, phages that cannot are called *virulent*. Finally, under special conditions one can obtain and perpetuate phage particles that can lysogenize but have lost the power to complete the lytic cycle of phage growth Such phages cannot reproduce themselves but give rise to so-called *defective* lysogenic cultures.

In medical contexts one thinks of virulence as a viral property subject to gain and loss, and phages possess comparable properties Perhaps unfortunately, the property called virulence in phages is not analogous to these; it is better to think of the temperate character as something that can be gained and lost. The term "virulence" refers to a specific defect in phage function, just as the term "defective" refers to another

The lytic and the lysogenic cycles are very different phases of viral growth, and much of this chapter will be devoted to their separate description During the lytic cycle, something having properties suggestive of a phage chromosome, and lacking many properties of phage particles, multiplies This something is called *vegetative phage*. During the lysogenic cycle, something having similar properties multiplies in a different way This something is called *prophage*. Mature phage particles are formed only during the lytic cycle, and these evidently contain something having the properties of a phage chromosome It is assumed, and experiment confirms, that the multiplication of vegetative phage, like the multiplication of prophage, precedes and is independent of a separate process, called *maturation*, giving rise to the mature particles

Phage growth can be thought of as a single cycle with a bypass, composed of at least 3 stages and 7 processes, as summarized in Figure 43. It can also be thought of as 2 alternative cycles, and this view is useful because phages confined to either one or the other are known, as mentioned above. In the following pages we present the lytic cycle first and describe lysogeny only after a large part of the biology of phage has been discussed This presentation is illogical but seems

to be simplest and has some historical justification.

RESTING PHAGE PARTICLES

Infectivity. Phage particles can be enumerated by various physical, chemical and biologic methods, of which only two need be mentioned here *Infective particles* are counted in terms of the number of clearings or *plaques* to which they give rise when introduced into an agar layer heavily seeded with bacteria sensitive to infection By suitable variations in technic one counts in this way free phage particles, infected bacteria, lysogenic bacteria, lysogenizing phage particles, or particles giving rise to lytic infection The total number of particles can be counted by quantitative electron microscopy In favorable instances from 50 to 100 per cent of the visible particles form plaques (Luria et al, 1951, Kellenberger and Arber, 1957)

Morphology. Probably all phages, with the possible exception of the smallest ones which have not yet been identified in electron micrographs, form tadpole-shaped particles (Williams and Fraser, 1953) The coliphage T2, one of the largest, consists of a polyhedral head, hexagonal in cross section, and a cylindrical tail In frozen-dried preparations, the head measures 65×95 mμ, and the tail 25×100 mμ The particles can be emptied of nucleic acid by osmotic shock, leaving a protein ghost closely resembling the intact particle in form (Anderson, 1953, Herriott, 1951) The phage particle consists, therefore, of a protein shell inside which the nucleic acid is rather tightly packed Micrographs are shown in Chapter 2

The tail of the T2 particle consists of a central pin running full length, surrounded by a tubular sheath (Kellenberger and Arber, 1955, Williams and Fraser, 1956) At the end of the tail are attached several fibrils usually visible only as a terminal thickening The manner of attachment of the fibrils is not clear The staphylococcal phage K probably is constructed in a manner similar to T2 (Hotchin, 1954)

Many other phages differ in size, shape and probably in structural detail, but all must be made along sufficiently similar lines to perform the common task, attachment to and puncture of the bacterial cell, and injection of nucleic acid Little is known about the smallest phages, but they probably measure about 20 mμ in diameter and contain only 1 3 to 13 per cent as much DNA per particle as T2 (Tessman et al, 1957)

Chemical Composition. All phages that have been examined, probably including one of the smallest (Sinsheimer, 1957), are composed chiefly of protein and DNA Detailed information is at hand only for T2, which contains approximately equal amounts of these two substances (2×10^{-16} Gm of each per particle) and little else Fractionation by osmotic shock is of special value, because it separates the particles into intact ghosts (empty protein shells) and nucleic acid This permits in principle the isolation of DNA, together with its possible chemical associates By combining this and other technics, an upper limit to the associates has been defined (Hershey, 1957b) The ghosts account for about 95 per cent of the organic material other than DNA The remainder of presumably internal constituents includes a small amount of a typical protein, a low molecular weight polypeptide of simple composition, one or two unidentified free amino acids, and two purine derivatives None of these minor components is well characterized, but each is distinct from the others None appears to be closely associated with the DNA

Antigenic Structure. All or nearly all phages are good antigens, and distinctly different phages are antigenically unrelated to each other They are always antigenically unrelated to their hosts Independently isolated phages obviously similar in other respects tend to be similar, but not identical, by serologic criteria Two phages differing only by one or two mutational steps are often serologically identical

Phage T2 presents at least two distinct surface antigens, apparently corresponding to head and tail proteins (Lanni and Lanni, 1953) Only the antibody directed against tail parts can neutralize the infectivity of phage particles Antibody against heads is detected by complement fixation Three functionally different types of antitail antibody have been found in serum prepared by immunization with T4, a coliphage similar to

T2 (Jerne, 1956, Jerne and Avegno, 1956) Therefore, different tail parts may correspond to different antigens. In addition, one can detect the usual multiple antigenic specificity by studying cross reactions. For example, T2 and T4 exhibit both common and specific antigenic determinants.

Phage T2 contains also an internal antigen, detectable only after osmotic shock, that is probably different from any in T4 or T6 (L Levine et al., 1957)

INITIAL STEPS OF INFECTION

The initial steps of infection include attachment of phage to bacterium and injection It is probably safe to assume that these steps are the same in lytic infection and in lysogenization, particularly since the decision between these alternatives can be influenced by experimental treatments applied some time after infection.

Primary Attachment. Several phages, perhaps all, attach to bacteria by the tips of their tails (Anderson, 1953) The attachment is exceedingly specific and determines, in part, the strains and the species of bacteria that a given phage can infect The primary reaction occurs between the attachment organ of the phage particle and receptor substances, often major antigens, present in the bacterial cell wall (Kellenberger and Arber, 1955, Weidel and Kellenberger, 1955, Jesaitis and Goebel, 1955). In T2, the terminal fibrils in the tail act as cementing substance (Williams and Fraser, 1956), and the tail pin punctures the cell wall (Kellenberger and Arber, 1955), apparently aided by an enzyme hidden in the tail (Brown and Kozloff, 1957) The attachment reaction also stimulates release of DNA (Jesaitis and Goebel, 1955), and the whole process is mimicked when T2 reacts with zinc complexes in solution (Kozloff et al, 1957) The interacting factors seem to be, tail fibrils and receptor substance; zinc protein in the cell wall and thiolester bonds holding the tail parts together (Kozloff and Lute, 1957); tail enzyme and bacterial substrate (not receptor substance) (Koch and Weidel, 1956)

In addition, phage-specific cofactors, chiefly various cations, must be present in the medium if attachment is to occur at all (reviewed by Tolmach, 1957). It is generally assumed that specific cation requirements imply specific effects in addition to those attributable to the reduction of electrostatic barriers, but clear evidence for this has not been obtained (Beumer et al, 1957). However, one such cofactor, L-tryptophan, required for the attachment of certain strains of T4, is thought to alter tail structure, possibly causing extension of tail fibrils in a process akin to reversible denaturation of proteins (Sato, 1956; Cheng, 1956) A pH-dependent, reversible alteration in the structure of T2, detected so far only by hydrodynamic methods (Taylor et al, 1955), suggests that one role of cations may be similar to that indicated for tryptophan

Injection. Injection of DNA, as a manner of penetration of phage into bacterium, can be demonstrated very simply by appropriate methods of electron-microscopy (Anderson, 1953) As an idea, it occurred more or less simultaneously to several people The first convincing experiment was performed by Hershey and Chase (1952) They prepared phage particles labeled in their DNA with radiophosphorus, or in their proteins with radiosulfur If either type of preparation was mixed with bacteria to permit attachment of phage particles and centrifuged, most of the radioactivity went down with the bacteria. This is, in fact, one useful method of measuring attachment of phage to bacteria If, however, the suspension of infected bacteria was first spun in a Waring blendor and then centrifuged, labeled DNA went down as before, but 80 per cent of the labeled phage protein was left in the supernatant fluid The infected bacteria submitted to this treatment remained competent to yield phage. The proper interpretation of these results called for additional experiments but will be obvious to the reader from the facts already summarized

The conditions specifically requisite for injection are not yet elucidated. Calcium ions are required for injection by T5 (Luria and Steiner, 1954). Metal chelators interfere with injection of T2 (Kozloff and Lute, 1957) T2 inactivated by dilute formaldehyde or by ionizing radiations does not inject efficiently but perhaps does not attach normally either (Hershey and Chase, 1952, Hershey et al, 1954). Dependence on bacterial metabolism is

suggested by the effects ("abortive infection") of such agencies as starvation, cyanide and low temperature (reviewed by Gots, 1958).

The question of what is injected, besides DNA, has been investigated in some detail (Hershey, 1957b) The detected substances include all or most of the minor components mentioned above: a typical soluble protein, a simple peptide, one or two free amino acids, and other trace substances probably too small in amount to be interesting The most massive fraction, the typical protein, weighs about 10^{-17} Gm per phage particle, or 4 per cent of the weight of the DNA In addition, however, the tail pin probably penetrates the cell wall, and its role in infection is not known Some of the tail parts presumably account for much of the 15 or 20 per cent of the protein of T2 that cannot be removed from infected cells by spinning in the blendor.

Once the cell walls of bacteria have been damaged, the "protoplasts" can be infected by degraded phage particles, possibly by phage DNA alone (Spizizen, 1957; D Fraser et al, 1957). Thus a large part of the injection apparatus, and its rigidly specific character, are concerned with penetration of the cell wall

INFERENCES I

The diversity of size and shape among particles of different phages, more or less correlated with variations in other properties such as antigenic structure and host specificity, provides a preliminary basis for recognizing diverse species and other taxonomic groups (Adams, 1958) This conclusion constitutes one half of the meaning of the word virus The other half resides in the chemical and functional simplicity of the resting particles and especially that of the multiplying particles to be described presently The two halves evidently comprise the whole of any general notion about viruses and show quite clearly how phages differ from other things subject to biologic classification

The manner in which infection occurs, by attachment at the cell surface and penetration of DNA, shows that most of the viral protein serves solely as a protective device and delivery mechanism for the DNA Only the latter, in co-operation with the bacterium and a few minor viral components of unknown use, can function directly to initiate viral growth. The suggestion is obvious that the phage chromosome as it occurs in the resting phage particle may be composed solely or mainly of DNA

Injection is a formidable task and, in T2, calls for an elaborate apparatus In resting phage particles, as in more complex organisms, the most complicated structures seem to be devoted to the most specialized tasks, and indeed this ought to be so, since stripped to essentials all things that grow must be very much alike At any rate it is with this optimistic hope that we turn to the consideration of other stages in the life cycle of phages

THE LYTIC CYCLE

Multiplication of Vegetative Phage. The term "vegetative phage" originally referred to an idea—that there existed an unknown, noninfective form in which phages multiply. It soon became evident that one must distinguish between multiplication in the lytic cycle and multiplication in lysogenic bacteria, hence, the terms "vegetative phage" and "prophage" A more exclusive definition is now possible and will better serve present needs *Vegetative phage is the structure whose production during the lytic cycle of phage growth coincides with the accumulation of the chromosomes of future phage particles* This definition is intended to exclude (1) all structures not essential to the replication of chromosomes and (2) all structures, whatever their role in replication, that are not incorporated into the final particles

The following experiments, important also for an understanding of phage growth in general, furnish the proof of existence of vegetative phage as defined above. In essence they are variations of the one-step growth experiment of Ellis and Delbruck (1939) in which one measures, instead of a total yield of phage released by spontaneous lysis of infected cells, the partial yields released by artificial lysis at various earlier times Such experiments, first described by Doermann (1952 and earlier) and Anderson and Doermann (1952) are performed as follows.

Bacteria are infected with a measured ratio of phage particles to bacteria, for example 1 to 10 to produce *single infections*, or 5 to 1

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and how it is replicated. These questions present themselves mainly in chemical terms and meet with the difficulty that only genetic methods, as in Doermann's experiments, can identify vegetative phage. Before this difficulty could be overcome, much purely chemical investigation, begun by Cohen and pursued by numerous workers (Chap 3), was necessary. A few results of this work can be summarized as follows

In *Escherichia coli* infected with T2, all the DNA synthesized contains hydroxymethylcytosine and is phage precursor No bacterial DNA (containing cytosine) is formed Radiophosphorus incorporated into DNA subsequently appears in phage particles. The kinetics of the transfer process show that the average cell contains about 45 phage-equivalent units (2×10^{-16} Gm each) of DNA, much of it in a single metabolic pool to which 5 units per bacterium per minute are added by synthesis and from which 5 units per bacterium per minute are removed to make phage particles Moreover, if maturation of phage particles is prevented by inhibiting protein synthesis, the pool of phage precursor DNA can be built up to at least 3 times the normal size and yet function with normal kinetics if protein synthesis is subsequently allowed to resume. In this manner one can obtain phage particles whose DNA and protein have been synthesized at different times, showing that synthesis of phage precursor DNA is independent of synthesis of phage precursor protein (Hershey and Melechen, 1957)

In the experiments last mentioned, chloramphenicol was used to inhibit protein synthesis, and phage precursor DNA accumulated in the presence of that substance can be called "chloramphenicol DNA." One of the first questions about the chemistry of vegetative phage can then be put very simply Is chloramphenicol DNA and vegetative phage the same thing?

To answer this question it is necessary to determine whether chloramphenicol DNA already possesses significant properties in common with the chromosomes of resting phage particles. Of the several methods available for this test, only one has been applied successfully It depends on the well-supported hypothesis that ultraviolet light produces its characteristic effects on phage particles by directly damaging the chromosomal substance These effects, and evidence for the hypothesis, will be described in the section in this chapter called radiation genetics.

Tomizawa (1958) found that irradiation of chloramphenicol DNA in infected bacteria, followed by removal of chloramphenicol, leads to the production of noninfective phage particles possessing unmistakably the properties exhibited by phage particles that have themselves been irradiated. The number and the proportion of dead particles in the yield depends directly on the amount of phage precursor DNA present in the cells at the time of irradiation, showing that the photochemical lesions do not themselves replicate. If the amount of irradiated DNA is large, the yield consists almost exclusively of noninfective particles, a result consistent with the radiochemical finding that, when the amount of chloramphenicol DNA is large, the yield consists of particles containing almost exclusively chloramphenicol DNA. Irradiation before infection of the bacterial DNA, which is an indirect precursor of phage particles, does not cause the production of noninfective particles containing the phosphorus of the irradiated DNA Pending confirmation by other methods, these results can be taken as proof that vegetative phage, and therefore the chromosomes of phage particles themselves, are composed of DNA to the exclusion of all other known phage constituents (Hershey, 1957b)

The experiments just described, if correctly interpreted, establish a direct correlation between the synthesis of phage precursor DNA and the accumulation of radiation-sensitive chromosomes and therefore provide two methods for measuring the multiplication of vegetative phage Judging by the relatively precise chemical data (Hershey and Melechen, 1957) vegetative replication begins from 5 to 10 minutes after infection, soon reaches a constant rate of production of about 5 vegetative particles per bacterium per minute and produces a population containing a constant number of vegetative particles by the 20th minute However, the actual size of this population is unknown The average cell contains about 45 phage equivalent units of phage precursor DNA of which an uncertain fraction is vegetative phage and the remainder must be con-

to produce *multiple infections.* A few minutes later, any unadsorbed phage particles from the original input having been eliminated by antiserum or centrifugation, the culture is diluted to contain only about 1,000 bacteria per cc, which prevents further interactions between phage particles and bacteria. Titration by means of the plaque count method made at this time shows one plaque corresponding to each infected bacterium Another sample of the diluted culture, to which cyanide is added to stop phage growth, is lysed by mechanical vibration or other means and then titrated. This sample yields only a negligible number of plaques arising from infected bacteria that survived the lysing treatment The result is understandable, since the infecting phage particles were destroyed in the act of injection, and insufficient time has elapsed for the formation of new particles

By titrating samples lysed in the same way at different times one observes an *eclipse period* before the first infective phage particle is produced in the cell This is followed by a linear increase in number of infective particles up to the time of spontaneous lysis, after which the number remains constant By comparing different methods of lysis Doermann found that cyanide alone is sufficient to induce lysis after the end of the eclipse period, and now the experiment is usually performed by simply adding cyanide or chloroform to the samples to be titrated. The eclipse period for different phages varies from a few minutes to half an hour and is usually half as long as the *latent period* measured from the time of infection to the commencement of spontaneous lysis

The experiment yields direct information only about intracellular accumulation of infective phage particles This occurs, for phage T2 at 37°, at a linear rate of 3 to 8 phage particles per bacterium per minute, beginning 10 to 15 minutes after infection and continuing, under special conditions in which spontaneous lysis can be postponed, for 2 hours or more, sometimes yielding more than 1,000 particles per bacterium

Doermann (1953 and earlier) applied this technic to bacteria infected with *two mutant* phages, h and r13 of T2, between which a low frequency of genetic recombination can be observed. Previous work had shown that the recombinants arising in this cross do not appear in large intrabacterial clones and therefore must be formed relatively late during multiplication of the phage (Hershey and Rotman, 1949) However, Doermann found that the first few phage particles formed already included both parental and recombinant types. Therefore, he postulated a structure, called vegetative phage, capable of multiplying and containing the genetic determinants of future phage particles but lacking the infective property of phage particles According to this hypothesis, infectivity would have to be acquired by a second process, called maturation, by which vegetative particles are converted into the well-known finished form

What is the significance of the period of eclipse? It could be supposed that this represents the minimum time for a vegetative particle to mature in an infected bacterium, in which case the intrabacterial population of unfinished particles would consist mainly of a series of intermediate forms between vegetative phage and the final product. Visconti and Garen (1953) found, on the contrary, that a phage particle infecting a bacterium in which phage growth was already under way could contribute genetic markers to phage particles finished only 4 minutes later. For this and other reasons it is now believed that an infected bacterium contains numerous vegetative particles and eventually finished particles but relatively few particles in intermediate stages, and that most of the eclipse period is devoted to the preparation for, and the actual process of, vegetative reproduction

How does multiplication take place? Luria (1951) showed that mutations occurring during vegetative growth produce branching mutant clones of phage in individual bacterial cells. Thus each vegetative particle seems to have the capacity to multiply. If so, multiplication is a geometric process, and this step, at least, in the production of phage particles does not obey the assembly line principle.

The multiplication of vegetative phage has not yet been observed in a direct, quantitative manner, but methods are being developed for this purpose as described below.

Synthesis of Phage-Precursor DNA Given the idea that the unit of vegetative reproduction is something different from the familiar phage particle, one asks what it is

serum (Maaløe and Symonds, 1953), most accurately after fractional centrifugation to separate them from phage particles (Hershey, unpublished). The serologically specific protein not contained in phage particles, measured in this way, can be called surplus antigen (Hershey, 1956).

Surplus antigen is first formed in the cells a few minutes before phage particles appear. The amount increases rapidly, reaching a constant level of about 15 phage equivalent units (2×10^{-19} Gm. of sulfur each) per bacterium by the 20th minute after infection. Only about half of this material can be sedimented under conditions that throw down empty phage membranes. Much or all of it seems to be phage precursor material, as shown in two ways. First, one can measure an upper limit to the total phage precursor protein present in the cells by an independent tracer method (Hershey and Melechen, 1957). This method shows that the amount of phage precursor protein is approximately equal to the amount of surplus antigen, about 15 units per bacterium. Second, one can observe the flow of radiosulfur out of surplus antigen and its simultaneous incorporation into phage particles (Hershey, unpublished). This result confirms that most of the surplus antigen is in fact phage precursor, and therefore that most of the phage precursor protein in the cells is serologically specific.

These facts raise the question of how the 15 units of phage precursor protein and the 45 units of phage precursor DNA are associated in the cell. In lysates, no association can be detected (Watanabe et al., 1954). However, there must be some DNA-containing intermediates in the assembly of phage particles (Hershey and Melechen, 1957). The failure to find them may mean that their number is small, requiring the assembly process to be extremely rapid, or that the intermediate forms are too fragile to survive the lysing procedures.

The phenomenon of phenotypic mixing described below shows that, in mixed infections with T2 and T4, the tail parts of a single phage particle can be determined by both viral lineages. This suggests that tail proteins and DNA are made separately and later incorporated into phage particles, but perhaps other interpretations are possible. It must be concluded that the manner of association between phage precursor DNA and phage precursor protein by which vegetative phage is converted into resting phage is unknown.

The separation of protein synthesis and DNA synthesis during phage growth has been accomplished in the reverse manner to that seen in experiments employing chloramphenicol (Watanabe, 1957). Once DNA synthesis has begun in infected cells, large doses of ultraviolet light applied to them suppresses further DNA synthesis much more strongly than it suppresses synthesis of serologically specific phage protein. Protein synthesis is thus, in principle, independent of DNA synthesis. Moreover, Tomizawa's (1958) experiments described earlier show that the whole process of maturation to produce noninfective phage particles with functionally complete membranes can occur in cells containing almost exclusively irradiated DNA. Therefore, maturation is independent of many DNA functions on which phage growth as a whole depends.

Lysis. Little is known about the actual lysis of phage-infected bacteria. As mentioned earlier, enzymes are found among the tail parts of T2 and T4. These act on cell walls and presumably aid penetration at the time of infection. They are probably responsible for the phenomenon known as lysis from without, which is seen with the phages mentioned and few others (Visconti, 1953).

Jacob et al. (1957) have found an enzyme having some of the properties of lysozyme in lysates of bacteria infected with phage lambda. Among various defective lysogenic cultures, it is produced only in those that lyse after induction with ultraviolet light. The lysing enzyme may represent a surplus of the same enzyme manufactured for phage tails, which would thus perform a dual role in phage growth.

INFERENCES II

The lytic cycle of phage growth is characterized best by summarizing both successes and failures of the line of investigation outlined above.

Following infection, the first process of known significance is the production of phage precursor DNA. This DNA has been identi-

tained in particles further along on the way to becoming finished phages The upper limit to the number of vegetative particles, 45, corresponds to an upper limit to the generation time, 9 minutes, if reproduction is really geometric

One may ask whether the linear rate of DNA synthesis reflects the fixed number of vegetative phage particles that the cells must contain This is unlikely, since the rate is similar and approximately linear in the presence of chloramphenicol, where all the precursor DNA accumulates as vegetative phage Evidently, the rate of synthesis is not determined by the number of vegetative particles, except possibly at early times

Another method for measuring vegetative reproduction has been applied to questions about defective lysogeny and will be cited in connection with that subject

Preparation for DNA Synthesis. When bacteria are infected with T2, synthesis of bacterial DNA stops abruptly, and synthesis of phage precursor DNA begins only after a delay of several minutes What is occurring during the period of delay? Numerous evidences show that synthesis of protein is essential during this period (Chap. 3). For example, in the experiments with chloramphenicol discussed above, it is necessary to wait from 5 to 10 minutes after infection before adding the antibiotic to permit resumption of DNA synthesis This result is perhaps not surprising, since at least one new enzyme, which hydroxymethylates cytosine, is formed and presumably is required for growth of T2 (Chap 3) For other phages analogous requirements can be imagined

Radiobiologic experiments go much further in suggesting that the phage DNA itself, though essential to initiate DNA synthesis, is superfluous thereafter (reviewed by Stent, 1958). For example, ultraviolet light, which produces lethal damages in the DNA of phage particles, has relatively little effect on the DNA-synthesizing capacity of infected bacteria (Watanabe, 1957). At one time such results were interpreted to mean that vegetative phage itself was resistant to ultraviolet light. Tomizawa's (1958) experiments described above show that this is not so; only the capacity of infected cells to produce vegetative phages is resistant.

One way of explaining the radiobiologic findings is to suppose that the infecting phage particle organizes a radioresistant structure, composed of protein for example, that in turn can regenerate phage chromosomes (Stent, 1958). According to this hypothesis the basic mechanism of DNA synthesis could hardly be geometric

Another way of explaining the radiobiologic findings is to suppose that one or several irradiated chromosomes somehow can regenerate undamaged ones without being repaired themselves, but only provided that they have performed certain critical tasks before irradiation (Benzer, 1952; Krieg, 1957) According to this hypothesis, the radiobiologic experiments suggest clues concerning genetic recombination and other functions of DNA but do not prohibit DNA from playing a direct role in its own synthesis

At the present time the two interpretations are about equally unsatisfactory, but it is necessary to consider how to distinguish between them and to admit that this major gap exists in our understanding of DNA synthesis and phage growth.

Maturation. We concluded above that the synthesis of phage precursor DNA and the production of vegetative phage particles are a single process, which is independent of synthesis of phage precursor protein It follows that maturation comprises the whole sequence of processes by which preformed phage DNA is incorporated into finished phage particles. This includes the complete synthesis of several proteins, their organization into characteristic phage membranes, and, no less mysterious, the packing of DNA inside them

In experiments employing chloramphenicol, it is clear that the synthesis of phage precursor DNA precedes the start of maturation. In the formation of individual phage particles this also must be the normal sequence of events (Hershey and Melechen, 1957) Considering the infected cell as a whole, however, the two processes normally occur side by side.

Lysates of bacteria infected with T2 contain, besides completed phage particles, other morphologically and serologically recognizable phage-specific structures (Chap 3). The protein content of such structures can be measured as radiosulfur precipitable by antiphage

serum (Maaløe and Symonds, 1953), most accurately after fractional centrifugation to separate them from phage particles (Hershey, unpublished) The serologically specific protein not contained in phage particles, measured in this way, can be called *surplus antigen* (Hershey, 1956).

Surplus antigen is first formed in the cells a few minutes before phage particles appear. The amount increases rapidly, reaching a constant level of about 15 phage equivalent units (2×10^{-15} Gm of sulfur each) per bacterium by the 20th minute after infection Only about half of this material can be sedimented under conditions that throw down empty phage membranes Much or all of it seems to be phage precursor material, as shown in two ways First, one can measure an upper limit to the total phage precursor protein present in the cells by an independent tracer method (Hershey and Melechen, 1957) This method shows that the amount of phage precursor protein is approximately equal to the amount of surplus antigen, about 15 units per bacterium Second, one can observe the flow of radiosulfur out of surplus antigen and its simultaneous incorporation into phage particles (Hershey, unpublished) This result confirms that most of the surplus antigen is in fact phage precursor, and therefore that most of the phage precursor protein in the cells is serologically specific.

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Following infection, the first process of known significance is the production of phage precursor DNA This DNA has been identi-

fied with reasonable assurance as the chromosomes of future phage particles. Its production is independent of the production of phage precursor proteins Therefore, it is necessary to recognize a unit of reproduction, called vegetative phage, that might be described as nascent phage precursor DNA, composed, apparently, of DNA only

The phage particle is formed by a second process, called maturation, that calls for complete synthesis of phage proteins, their organization into phage membranes, and the inclusion of preformed DNA inside them The phage DNA apparently exerts its control over this process in an indirect manner, since it is clear that the maturation of a given particle is not dominated solely by the chromosome included in it, indeed, the latter may have been severely damaged by ultraviolet light before maturation started.

During a steady state period of phage growth, the T2 infected cell contains enough phage precursor DNA to make 45 phage particles and enough phage precursor protein to make about 15 Both precursors are formed at a rate equal to the rate at which they are used to make phage particles, about 5 phage particle equivalents per bacterium per minute.

Present methods do not permit one to distinguish between phage precursor DNA present in vegetative phage and in hypothetical intermediates in the process of maturation For this reason the above figures cannot yet be related to the kinetics of growth of vegetative phage

No weighty evidence has been obtained as to whether or not each vegetative phage particle serves as a center for its further reproduction, but again the question has been framed in quasi-experimental terms

GENETIC STRUCTURE AND FUNCTION

Mutations. Any heritable change in the properties of a phage arising in an initially homogeneous line may be called a mutation. The observed mutations in phage affect such characters as host range, plaque type, growth potential, latent period, ability to lysogenize, sensitivity to high temperatures, and dependence on cofactors. These categories are not mutually exclusive. For example, mutations affecting ability to lysogenize are usually recognized as alterations in plaque type, and mutations affecting host range are often accompanied by changes in sensitivity to heating. Because of the ease of recognizing them, only mutations affecting host range or plaque type are commonly used in genetic experiments.

The mutations that have been studied best show a few characteristic features in common. They occur exclusively during the growth of the phage, producing mixed yields of the original and mutant types in single infected bacteria (Luria, 1951). Mutagenic agents such as ultraviolet light, proflavine and bromouracil increase the frequency of phage mutations, but these agents are demonstrably effective only when applied to the bacteria, before infection (Jacob, 1954; Fraser, 1957), after infection (Latarjet, 1949, DeMars, 1953, Litman and Pardee, 1956), or separately to phage and bacteria (Weigle, 1953, Weigle and Dulbecco, 1953)

When analyzed by recombination tests, mutational changes are revealed as local changes in chromosome structure, which usually occur singly but can be accumulated by successive independent steps (Hershey and Rotman, 1948)

The frequencies of repetition of identical mutations are seldom measurable but probably vary at least over the range between 10^{-3} and 10^{-10} per phage produced (Streisinger and Franklin, 1956, Benzer, 1957) The frequencies of repetition of nonidentical mutations of similar type depend chiefly on structural requirements for genetic function (Hershey and Rotman, 1948; Streisinger and Franklin, 1956, Benzer, 1957) Thus, the loss of a particular function may be very frequent because alternative structural changes at many points interfere with it. On the other hand, the restoration of a function recently lost by mutation often calls for reversal of the original change and may be rare or frequent, depending on the idiosyncrasies of the first mutation In such cases the distinction between gain and loss of function seems to have meaning, one can recognize a "positive" functional state as one requiring a highly specified genetic configuration (Streisinger and Franklin, 1956).

Genetic Recombination. When a bacterium is infected with one or more particles of each of two closely related phages, preferably two different mutants of the same phage species, one obtains a yield of phage containing particles of the two parental types, plus a certain number of particles that obviously are derived by recombination of characters present in the parental phages. The rules governing the kinds of recombination observed are extremely simple, and the qualitative results can be predicted, with rare exceptions, from the mutational history of the stocks.

Suppose, for example, one has isolated from T2 a host range mutant, called h, capable of lysing a bacterial strain, called B/2, resistant to infection by T2. Secondly, one has isolated a rapidly lysing mutant, called r, that differs from T2 with respect to type of plaque. T2 itself may then be called h^+r^+, meaning simply not-h and not-r. The genetic crossing between the two mutants is performed by infecting bacteria with both simultaneously and examining the yield of new phage particles. The first notable result is the appearance of new types of phage. The simplicity of the result resides in the fact that all the particles, like their parents, can be classified as h or h^+, and as r or r^+, with respect to hereditary character. This is the reason for concluding that individual mutations generally correspond to single changes in genetic structure. Therefore, the only possible combinations of the characters are h^+r^+, hr, h^+r, and hr^+. The rule of recombination deduced from the experiment merely states that when two dissimilar phages are crossed, all the anticipated recombinations of the characters present in the parents will be found among the offspring. Thus, bacteria infected with the first and the second or with the third and the fourth of the genotypes listed above will yield all 4. Bacteria infected with any other pair, for example the first and the third genotypes, will yield only the parental combinations. This is the main justification for speaking of genetic recombination between phages (reviewed by Hershey, 1953).

In crosses involving a 2-factor difference, as in the example cited, a *recombination frequency* can be measured very simply. It is the ratio between the number of recombinant phage particles and the number of phage particles of all kinds counted in a sample of the progeny from the cross. What determines this frequency? Experiment shows that a number of conditions are important which, however, can be divided into 2 classes. First, several kinetic variables affect recombination frequency in approximately the same way that time and concentrations affect the progress of a chemical reaction. These variables have been analyzed in an elegant theory by Visconti and Delbrück (1953). Second, if all other variables are held constant, one sees that recombination frequency is markedly dependent on the particular pair of mutants chosen for the experiments. Under these conditions one measures *linkage* between the markers under test. This measurement is of importance because it leads to information about the structure of the phage chromosome. In short, one can show that mutations occur at various places along the length of a linear chromosome (Hershey and Rotman, 1948), that a phage particle contains only one such chromosome (Jacob and Wollman, 1955), and that physiologically related mutations tend to occur within short segments of it (Benzer, 1955).

The main conclusion deriving from analysis of the kinetic variables is that genetic recombination must be interpreted as the consequence of repeated random matings in a population of, presumably, vegetative phage particles. There are about 5 mating cycles during the growth of T2 and T4 in one bacterium, whereas most other phages that have been studied mate only about once. The difference is unexplained and presents a major challenge to current thought about mechanisms of recombination (Stent, 1958).

Heterozygosis. It was stated above that a phage particle issuing from an h × r cross must be either h or h^+ and either r or r^+ in gentoype. This is not quite so, a few exceptional particles yield clones that are mixed with respect to one character or the other. Such particles are partial heterozygotes (Hershey and Chase, 1951), containing large chromosomal segments from both parents with short overlapping regions (Levinthal, 1954). All observers have realized that the heterozygotes must be related to the manner of production of recombinants. For the present,

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isolation of and discrimination among many similar mutants. Without attempting to describe here the technics, we summarize some typical conclusions (Benzer, 1955).

A certain class of r mutations in T4 occur in what is known as the *rII* region of the phage chromosome. This region is concerned with functions essential for the growth of T4 in a lysogenic derivative of *E. coli* K12, but not essential for growth in its nonlysogenic derivatives. The *rII* region can be subdivided by complementarity tests into 2 functionally distinct segments lying adjacent to each other, each segment measuring only a few units in length. In each segment, many mutations can occur that at the same time give rise to the *r* character and restrict the growth potential. The least distance between adjacent mutations separable by recombination measures only 0.02 units. The whole chromosome of T4 is 200 or more units long. If the unit of length is taken at face value, the whole chromosome contains at least 200/0.02 or 10,000 recombination points. Among the internal constituents of the phage particle, 2 only contain this many "joints." The DNA consists of about 200,000 nucleotide pairs, the internal protein contains about 35,000 amino acid residues. The computation evidently permits, but does not require, the chromosome to be composed of DNA. The remarkable feature of this work lies in the detail with which the functional topology of the phage chromosome, and of its mutational sites and combining units, can be described. On the one hand, this permits thorough testing of the fidelity of the description. On the other hand, it should facilitate future analysis of DNA function in physiologic and chemical terms.

Radiation Genetics. Irradiated phage particles possess diverse properties depending on the kind, the amount and the conditions of irradiation, the kind of phage and the conditions of test (Watson, 1952, Bowen, 1953). At the present time the effects of ultraviolet light and of disintegration of incorporated radiophosphorus are especially interesting because of many reasons for believing that these radiations produce mainly damages localized in the phage DNA. Genetic experiments with irradiated phages confirm this belief and yield further clues to DNA function.

Phages T2 and T4 "killed" by ultraviolet light have been studied most thoroughly (reviewed by Bowen, 1953). Bacteria infected with a single particle of such phages are themselves killed, but growth of the phage is blocked at an early step, as shown by failure of DNA synthesis (Vidaver and Kozloff, 1957). The irradiated particles show a number of remarkable properties, some of which can be described briefly as follows. *Photoreactivation* is observed by exposing bacteria infected with the irradiated particles to visible light. This causes phage growth to proceed in some of the bacteria (Dulbecco, 1955). The quantitative results for T2 can be interpreted to mean that 56 per cent of the damages produced by ultraviolet light are repaired in illuminated bacteria. *Multiplicity reactivation* can be observed by infecting bacteria with more than one irradiated particle. Some of the bacteria receiving several individually noninfective particles produce a viable phage progeny (Luria and Dulbecco, 1949). This effect can be interpreted to mean that two or more radiation-damaged particles sometimes cooperate to bypass the damages. *Cross reactivation* occurs when bacteria are infected with a mixture of irradiated and unirradiated particles. To observe it the particles must be genetically marked. Under these conditions the yields of phage from individual bacteria often show markers contributed by the irradiated phage particles. Quantitative experiments show that individual markers, not whole phage particles, are reactivated. For this reason the phenomenon is now often called *marker rescue* (Doermann et al., 1955).

Photoreactivation focuses attention on the nature of the photochemical damage produced by ultraviolet light and the manner in which

system (Goodgal et al., 1957), it is probable that the damages are directly repaired (see also Lennox et al., 1954). The chemistry is obscure.

Cross reactivation and multiplicity reactivation are thought to occur not by chemical restoration of damaged DNA but by genetic reassortment of undamaged parts (Doermann et al., 1955). For the present, therefore, one can ignore the chemistry and focus on genetic mechanisms. Cross reactivation may reflect in

however, they serve mainly to point up our ignorance of how recombination really occurs.

Interspecific Crosses. The recombination method permits one to analyze genetic differences between two closely related phage species, thus making available differences that cannot be observed among mutants of the same phage For example, T2 and T4 differ serologically. In crosses between them, this difference segregates as a single factor that controls both serologic specificity and specificity of attachment to bacteria (Streisinger, 1956a) This result tends to show that both properties should be referred to the same component of the tail of the phage particle.

T2 and T4 also differ with respect to the glucose content of their DNA (Sinsheimer, 1956; Jesaitis, 1957). This difference does not readily segregate in crosses; all particles tend to resemble the T4 parent in glucose content and certain other properties (Streisinger and Weigle, 1956) It is not clear whether this result signifies an unusual kind of inheritance or merely reflects one or another complication likely to be encountered in interspecific crosses

Phenotypic Mixing. When bacteria are infected with two phages differing mainly or exclusively in host range, for example T2 and T4, many particles in the progeny exhibit an abnormal host range specificity. The abnormality may be described as lack of correspondence between genotype and phenotype (Novick and Szilard, 1951). Not only do such particles fail to breed true to type, but some of them exhibit a mixed phenotype, with both T2-like and T4-like character. As far as known, only the host range character, and the related serologic specificity and sensitivity to heat, are affected (Streisinger, 1956b). Evidently, this means that in a mixed infection the character of the tail protein of a given phage particle is often determined not by the genetic material in that particle but by the action of viral genes present in the cell as a whole. It remains to be learned whether or not this is a general characteristic of the protein structures of phage particles, and at what level of organization it makes its appearance.

Chromosome Structure. The recombination tests by which one measures linkage between different mutational markers in the phage chromosome yield information principally about distances between markers, and order of arrangement. Genetic map distances are measured in recombination units, the unit of length being 1 per cent recombination frequency. Map distances are assumed to be related more or less directly to physical distances. Order of arrangement is inferred from the following principle. If 3 markers are linked in the order abc, separation of b from the other two by recombination will be a relatively rare event Linearity of structure, now established with great precision for small distances, is inferred from the additivity of distances and their consistency with tests of order.

The precision of the genetic analysis of chromosome structure depends chiefly on the number of mutations that can be discriminated by recombination tests. However, the power of the analysis lies in the possibility of recognizing mutations affecting different chromosomal functions The latter is accomplished (1) in terms of the visible effects of the different mutations and (2) by the test of complementarity If 2 phage particles exhibiting similar but nonidentical mutations can carry out, in mixed infection, a function of which each alone is incapable, the mutations are said to affect different functions If the 2 particles fail, their mutations affect the same function This method of analysis identifies a functionally unitary region of the chromosome only to the extent that the results of complementarity tests, the visible effects of the mutations concerned, and their disposition on the genetic map yield consistent interpretations In addition, one now expects a loss of function resulting from mutation to be made good only by reversal of the original mutation, potentially a rare event (Streisinger and Franklin, 1956). These principles, excepting perhaps the last mentioned, are not new, they form part of the classic tradition of genetics. The remarkable current development is their more or less thorough validation for 9 functionally unitary segments in chromosomes of 4 different phages, a result by no means anticipated by the workers concerned (Benzer, 1955, 1957; Streisinger and Franklin, 1956, M. Levine, 1957; Kaiser, 1957). Needless to say, these successes depend partly on the discovery of favorable materials and partly on technical inventions permitting the

isolation of and discrimination among many similar mutants Without attempting to describe here the technics, we summarize some typical conclusions (Benzer, 1955)

A certain class of r mutations in T4 occur in what is known as the *rII region* of the phage chromosome This region is concerned with functions essential for the growth of T4 in a lysogenic derivative of *E coli* K12, but not essential for growth in its nonlysogenic derivatives The rII region can be subdivided by complementarity tests into 2 functionally distinct segments lying adjacent to each other, each segment measuring only a few units in length In each segment, many mutations can occur that at the same time give rise to the r character and restrict the growth potential. The least distance between adjacent mutations separable by recombination measures only 0 02 units The whole chromosome of T4 is 200 or more units long If the unit of length is taken at face value, the whole chromosome contains at least 200/0 02 or 10,000 recombination points Among the internal constituents of the phage particle, 2 only contain this many "joints" The DNA consists of about 200,000 nucleotide pairs, the internal protein contains about 35,000 amino acid residues The computation evidently permits, but does not require, the chromosome to be composed of DNA The remarkable feature of this work lies in the detail with which the functional topology of the phage chromosome, and of its mutational sites and combining units, can be described On the one hand, this permits thorough testing of the fidelity of the description On the other hand, it should facilitate future analysis of DNA function in physiologic and chemical terms

Radiation Genetics Irradiated phage particles possess diverse properties depending on the kind the amount and the conditions of irradiation, the kind of phage and the conditions of test (Watson, 1952, Bowen, 1953) At the present time the effects of ultraviolet light and of disintegration of incorporated radiophosphorus are especially interesting because of many reasons for believing that these radiations produce mainly damages localized in the phage DNA Genetic experiments with irradiated phages confirm this belief and yield further clues to DNA function

Phages T2 and T4 "killed" by ultraviolet light have been studied most thoroughly (reviewed by Bowen, 1953). Bacteria infected with a single particle of such phages are themselves killed, but growth of the phage is blocked at an early step, as shown by failure of DNA synthesis (Vidaver and Kozloff, 1957). The irradiated particles show a number of remarkable properties, some of which can be described briefly as follows. *Photoreactivation* is observed by exposing bacteria infected with the irradiated particles to visible light. This causes phage growth to proceed in some of the bacteria (Dulbecco, 1955) The quantitative results for T2 can be interpreted to mean that 56 per cent of the damages produced by ultraviolet light are repaired in illuminated bacteria *Multiplicity reactivation* can be observed by infecting bacteria with more than one irradiated particle Some of the bacteria receiving several individually noninfective particles produce a viable phage progeny (Luria and Dulbecco, 1949) This effect can be interpreted to mean that two or more radiation-damaged particles sometimes cooperate to bypass the damages *Cross reactivation* occurs when bacteria are infected with a mixture of irradiated and unirradiated particles To observe it the particles must be genetically marked Under these conditions the yields of phage from individual bacteria often show markers contributed by the irradiated phage particles. Quantitative experiments show that individual markers, not whole phage particles, are reactivated For this reason the phenomenon is now often called *marker rescue* (Doermann et al, 1955)

Photoreactivation focuses attention on the nature of the photochemical damage produced by ultraviolet light and the manner in which it is repaired or bypassed by cellular processes aided by visible light Since photoreactivation of isolated DNA has been observed in another system (Goodgal et al, 1957), it is probable that the damages are directly repaired (see also Lennox et al, 1954) The chemistry is obscure

Cross reactivation and multiplicity reactivation are thought to occur not by chemical restoration of damaged DNA but by genetic reassortment of undamaged parts (Doermann et al, 1955) For the present, therefore, one can ignore the chemistry and focus on genetic mechanisms Cross reactivation may reflect in

part normal mechanisms of genetic recombination, in part a secondary effect of ultraviolet light that increases the frequency of recombination. In both T2 and lambda phages the second effect can be demonstrated directly in genetic crosses, even, in phage lambda, by doses of ultraviolet light too small to produce appreciable inactivation of phage particles (Jacob and Wollman, 1955).

The results of quantitative marker rescue experiments can be interpreted in the following way (Krieg, 1957). Since even small doses of ultraviolet light cause apparent marker inactivation, radiation damages at a considerable distance from the marker being tested can prevent rescue In fact, the apparent target size of a single marker for small doses of light is 8 to 10 per cent of that for killing of the whole phage particle. This presumably means that a given marker in particles receiving 10 lethal damages of radiation is potentially rescuable but has only about an even chance of being rescued or not under the given conditions of test. As the dosage of radiation is increased, the sensitivity of the surviving markers decreases, reaching, at very high doses, an apparent target size that is only about 0 7 per cent of that for primary lethal effects The potentially rescuable markers surviving high doses are rescued with a probability of only 0 02. The effects of increasing dosage are understood as follows It is supposed that, once a given marker has been bracketed by radiation damages, further damages are without effect unless they fall within the bracket This implies that the probability of rescue is determined mainly by the size of the bracket and vanishes at a certain minimum size.

The results so far described could perhaps be interpreted in different ways, without invoking persistent, localized damages to genetic material, or rescue by genetic recombination. However, two additional experiments support the orignal interpretation.

Hershey and Burgi (1956) showed by tracer experiments that the offspring from a [illegible]

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from the irradiated parent This shows that the radiation damages persist during infection and remain associated with the DNA of the irradiated particles.

Krieg (1957) correlated the inactivation by ultraviolet light of a specific chromosomal function with the loss of a genetic marker lying in the appropriate chromosomal region Recall that in a mixed infection with T4r$^+$ particles and rII mutant particles, both phages can multiply in a lysogenic derivative of *E. coli* K12, the genetic defect of the mutant particles being made good by the competence of the others. If the mixed infection is brought about with irradiated T4r$^+$ particles and unirradiated mutant particles, some bacteria yield phage, and some do not Among the individual yields, Krieg found a high frequency of r$^+$ marker rescues, as compared with rescues of another marker lying outside the rII chromosomal region However, in identical experiments employing a nonlysogenic strain of *E coli*, the two markers are rescued with equal frequency These experiments reveal a radiation-sensitive structure in T4 that is specifically required for growth in lysogenic K12, whose function tends to be destroyed simultaneously with losses of the r$^+$ marker. This structure is evidently the rII region of the phage chromosome identified by Benzer (1955). It follows that the radiation damages detected in marker rescue experiments are in fact chromosomal damages, and that the probabilty of rescue is determined in part by the chromosomal distances between the sites of damage and the marker under test Other evidence suggests that a large fraction, though perhaps not all, of the damages produced in phage particles by ultraviolet light are of this type (Doermann et al , 1955)

Marker rescue is also observed in comparable experiments with phage inactivated by decay of assimilated radiophosphorus (Stent, 1953; Stahl, 1956). Since the phosphorus in phage particles is contained in DNA, the damages must be localized in DNA (Stent and Fuerst, 1955), which means, at least in part, chromosomal damages The P^{32} decay method has been used in many ingenious ways, chiefly by Stent and his collaborators, to answer questions about DNA structure and function Some of these are mentioned in appropriate contexts in this chapter. In general, though not always in detail, the results parallel those obtained by comparable experiments using

ultraviolet light, confirming that both agents act primarily on DNA to produce chromosomal lesions

Transfer of Nucleic Acid from Parental to Offspring Phage. Putnam and Kozloff (1950) showed that labeled phosphorus contained in a parental generation of phage particles reappeared among the offspring particles. This suggested that one of the ways of learning something about how DNA performs its work would be to look at the mechanism of transfer The initial questions have now been answered with reasonable certainty (reviewed by Hershey and Burgi, 1956; Hershey, 1957b) Only DNA is transferred The transfer is potentially highly efficient It occurs, at least in part, in the form of large functionally specific pieces Therefore, by the appropriate use of isotopes it is possible to examine molecules of DNA before and after they have performed their multiple functions

The evidence for transfer of DNA in functional form is 4-fold Decay of transferred radiophosphorus occurring in the offspring particles can cause their death (Stent and Jerne, 1955) Some of the transferred pieces are very large (Levinthal, 1956) In genetic crosses, some of the transferred radiophosphorus remains preferentially associated with the genotype of the radioactive parent (Hershey and Burgi, 1956) In mixed infections with irradiated phage, some of the offspring particles receiving DNA from the irradiated parent are in consequence noninfective (Hershey and Burgi, 1956)

If radiations produce chromosomal lesions, as previously concluded, the transfer experiments reveal transfer of chromosomal DNA In addition, some of the experiments seem to show that part of the transferred DNA, at least, is not subject to progressive fragmentation during growth (Levinthal, 1956, Delbruck and Stent, 1957)

These solid gains are overshadowed at the moment by a new problem arising partly from the experiments cited It now appears that the DNA of T2 is composed of 2 fractions differing in molecular weight (Levinthal, 1956), in chemical composition (Brown and Martin, 1956) and in sensitivity to ultraviolet light (Hershey and Burgi, 1956) Some of the evidence for distinct fractions is purely formal, and all of it is rather preliminary. However, the question is raised whether phage particles may not contain both chromosomal and nonchromosomal DNA. Until this question is decided, the proper interpretation of the transfer experiments, and what clues they can yield concerning DNA function, remain obscure A related area of ignorance should be kept in mind Is the chromosome of T2 a single linear molecule of DNA or a bundle of molecules?

INFERENCES III

The particle of several phage species contains a single linear chromosome differentiated along its length into segments each of which has its own prescribed function Each segment is subject to mutation and is further subdivisible by genetic recombination at many points The units of physiologic action span only a small length of chromosome and appear to be continuous and nonoverlapping There is a strong suggestion that adjacent units of action tend to have related functions This manner of organization presumably reflects requirements for the concerted replication of segments and to a lesser extent requirements for concerted physiologic action Whatever the requirements for integration may be, it appears to be useful and permissible for the present to think of different segments as performing their functions independently of other segments

The analysis of these functions in chemical terms is all to come Some appreciation of the missing knowledge may be gained by comparing a typical problem in chemical genetics with a typical problem in pharmacology; the action of the simplest drugs on cells is seldom understood The power of genetic analysis, well illustrated by the work with phage, lies in its ability to circumvent chemical ignorance Of course, this does not justify chemical ignorance

The information about the phage chromosome is broadly similar to information about chromosomes in general The special interest in the phage chromosome derives from its small size and from evidence that it is composed solely of DNA, perhaps a single molecule of DNA. What is this evidence?

All of it, so far, begins with the proof that

part normal mechanisms of genetic recombination, in part a secondary effect of ultraviolet light that increases the frequency of recombination. In both T2 and lambda phages the second effect can be demonstrated directly in genetic crosses, even, in phage lambda, by doses of ultraviolet light too small to produce appreciable inactivation of phage particles (Jacob and Wollman, 1955).

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The results so far described could perhaps be interpreted in different ways, without invoking persistent, localized damages to genetic material, or rescue by genetic recombination. However, two additional experiments support the orignal interpretation.

Hershey and Burgi (1956) showed by tracer experiments that the offspring from a mixed infection with irradiated and live phage particles includes a few noninfective particles containing large pieces of DNA transmitted from the irradiated parent. This shows that the radiation damages persist during infection and remain associated with the DNA of the irradiated particles.

Krieg (1957) correlated the inactivation by ultraviolet light of a specific chromosomal function with the loss of a genetic marker lying in the appropriate chromosomal region. Recall that in a mixed infection with T4r$^+$ particles and rII mutant particles, both phages can multiply in a lysogenic derivative of *E. coli* K12, the genetic defect of the mutant particles being made good by the competence of the others. If the mixed infection is brought about with irradiated T4r$^+$ particles and unirradiated mutant particles, some bacteria yield phage, and some do not. Among the individual yields, Krieg found a high frequency of r$^+$ marker rescues, as compared with rescues of another marker lying outside the rII chromosomal region. However, in identical experiments employing a nonlysogenic strain of *E. coli*, the two markers are rescued with equal frequency. These experiments reveal a radiation-sensitive structure in T4 that is specifically required for growth in lysogenic K12, whose function tends to be destroyed simultaneously with losses of the r$^+$ marker. This structure is evidently the rII region of the phage chromosome identified by Benzer (1955). It follows that the radiation damages detected in marker rescue experiments are in fact chromosomal damages, and that the probability of rescue is determined in part by the chromosomal distances between the sites of damage and the marker under test. Other evidence suggests that a large fraction, though perhaps not all, of the damages produced in phage particles by ultraviolet light are of this type (Doermann et al., 1955).

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Immunity. From the facts just given, it appears that lysogenization, and hence the temperate character, depends first of all on some frequent early failure in the lytic cycle of phage growth In a *Salmonella* phage, at least, this can be attributed to the early production of an active immunity, not to an intrinsically feeble capacity to initiate vegetative reproduction (reviewed by Hershey, 1957a)

Once lysogeny is established, a very solid immunity to the carried phage is evident Lysogenic bacteria seldom lyse spontaneously, they do not lyse when reinfected with the phage they carry or with many of its relatives, nor do they as a rule become doubly lysogenic when similarly reinfected

It is plausible to attribute the immunity against double lysogenization to steric factors, if there is a characteristic site for each prophage a duplicate should be forbidden (Lwoff, 1953) The immunity to lytic development may also be related in some way to the prophage site Jacob and Wollman (1957) analyzed 14 temperate phages selected for their failure to show cross immunity. All of them proved to occupy different prophage sites in *E. coli* K12 This could mean either that immunity to lytic development has a steric basis (Lwoff, 1953) or that highly specific immune substances are involved (Bertani, 1956).

Induction. Exposure of some lysogenic bacteria to ultraviolet light sets in train the lytic cycle of phage growth in all or most of the cells At the same time the immunity to superinfection is abolished. Evidently, the inducing agent lifts the immunity in both senses The phenomenon is called induction and is reviewed by Jacob and Wollman (1953)

In a single species of bacterium, some prophages are inducible, and others are not In *E coli* K12, inducible prophages are found to occupy various sites in a cluster near the galactose fermentation markers of the bacterial chromosome In the same bacterium, noninducible prophages occupy sites in other regions A number of the inducible phages, although occupying different prophage sites and not showing cross immunity in lysogenic bacteria, are serologically related and genetically intercrossable (Jacob and Wollman, 1957) This correlation between phage properties and prophage region is unexpected and introduces new considerations into the difficult problem of phage taxonomy

Lysogenic Conversion. The presence of a prophage in a lysogenic culture affects bacterial properties in a variety of ways that are not always predictable Besides the propensity toward spontaneous and induced phage production, and the prophage specific immunity, several characters associated with the lysogenic state have been described Only two examples will be mentioned here

Burnet (1934) had noted a constant association of certain phages with specific bacterial types in *Salmonella* In a well-known example, lysogenization of certain bacterial species of group E1 (antigens 3, 10) with phages carried by group E2 converts the recipient bacteria into strains of group E2

ultraviolet light produces chromosomal lesions. From this point there are 3 lines of evidence. First, typical radiation sensitive structures accumulate in infected bacteria subjected to the action of chloramphenicol, which permits synthesis of phage DNA but prevents synthesis of all other important phage precursors. Second, DNA and typical radiation-sensitive structures, but no other important phage constituents, pass from parental to offspring phage particles during growth Third, ultraviolet light and decay of incorporated radiophosphorus produce similar effects on phage particles, whereas ionizing radiations produce more diverse effects Only DNA, among phage constituents, could show the pattern of radiosensitivity exhibited by phage chromosomes Efforts are being continued to make these 3 lines of evidence independent of each other, and a fourth one (Fraser et al, 1957) may be forthcoming If this proof is sustained, the information about chromosomal function outlined above is also information about function of DNA

THE LYSOGENIC CYCLE

Lysogeny A lysogenic bacterial culture is one in which the cells multiply normally but possess and transmit to their descendents the potentiality to produce bacteriophage This potentiality is seldom expressed under ordinary conditions, a typical lysogenic culture may yield phage in 1 cell per 10,000 per bacterial generation The rate of spontaneous production is necessarily low because the production of phage is lethal to the bacterium.

Apart from the few cells already on the way to lysis, lysogenic bacteria do not contain demonstrable phage particles. Therefore, it is concluded that lysogenic bacteria contain a phage-specific structure, called prophage, that serves as a hereditary determinant of lysogeny. Since prophage must contain a chromosome genetically equivalent to that of the resting phage particle on the one hand, and functionally comparable with other determinants of bacterial heredity on the other, it is natural to assume a common component for all three, namely, DNA.

Lysogenic strains of many bacterial species occur rather frequently in natural environments and may be more frequent than suspected, because often the lysogenic character must go unrecognized, owing to lack of means for detecting the carried phages

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Prophage. The concept of prophage is justified by the following evidence that a singly lysogenic bacterium differs from a comparable nonlysogenic one by the possession of a single phage-specific structure per bacterial nucleus, which occupies a chromosomal site that is characteristic for the carried phage.

1 Lysogenic cultures identical with the natural ones are readily produced by infecting bacteria with appropriate phages, as discussed below.

2 Lysogenic cultures, after exposure to radiation, occasionally yield nonlysogenic cells These can be reinfected to restore the lysogenic condition (Lederberg and Lederberg, 1953).

3. Lysogenic bacteria do not yield infective phage particles when lysed artificially (Burnet and McKie, 1929). Neither do they contain antigens characteristic of phage particles (Miller and Goebel, 1954).

4. Defective lysogenic bacteria can often be caused to lyse by ultraviolet light. In some instances the lysates do not contain any morphologically recognizable phagelike structures (Arber et al, 1957).

5 A number of different prophages have been established in *E coli* K12 Each of these proves, in crosses between lysogenic and nonlysogenic bacteria, to occupy a different site on the bacterial chromosome (Jacob and Wollman, 1957). In a strain of *Shigella*, the prophage P2 can occupy 3 different sites, but 1 of them is preferred (G Bertani, 1956). With some exceptions of uncertain significance, a lysogenic culture cannot carry 2 genetically marked but otherwise identical prophages

6. The expectation that prophage contains phosphorus is supported by experiments of Stent et al (1957) which suggest, in fact, that the prophage and the resting phage particle contain identical amounts of DNA, as determined by sensitivity to decay of incorporated radiophosphorus

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Immunity. From the facts just given, it appears that lysogenization, and hence the temperate character, depends first of all on some frequent early failure in the lytic cycle of phage growth. In a *Salmonella* phage, at least, this can be attributed to the early production of an active immunity, not to an intrinsically feeble capacity to initiate vegetative reproduction (reviewed by Hershey, 1957a).

Once lysogeny is established, a very solid immunity to the carried phage is evident. Lysogenic bacteria seldom lyse spontaneously; they do not lyse when reinfected with the phage they carry or with many of its relatives, nor do they as a rule become doubly lysogenic when similarly reinfected.

It is plausible to attribute the immunity against double lysogenization to steric factors; if there is a characteristic site for each prophage a duplicate should be forbidden (Lwoff, 1953). The immunity to lytic development may also be related in some way to the prophage site. Jacob and Wollman (1957) analyzed 14 temperate phages selected for their failure to show cross immunity. All of them proved to occupy different prophage sites in *E. coli* K12. This could mean either that immunity to lytic development has a steric basis (Lwoff, 1953) or that highly specific immune substances are involved (Bertani, 1956).

Induction. Exposure of some lysogenic bacteria to ultraviolet light sets in train the lytic cycle of phage growth in all or most of the cells. At the same time the immunity to superinfection is abolished. Evidently, the inducing agent lifts the immunity in both senses. The phenomenon is called induction and is reviewed by Jacob and Wollman (1953).

In a single species of bacterium, some prophages are inducible, and others are not. In *E. coli* K12, inducible prophages are found to occupy various sites in a cluster near the galactose fermentation markers of the bacterial chromosome. In the same bacterium, noninducible prophages occupy sites in other regions. A number of the inducible phages although occupying different prophage sites and not showing cross immunity in lysogenic bacteria, are serologically related and genetically intercrossable (Jacob and Wollman, 1957). This correlation between phage properties and prophage region is unexpected and introduces new considerations into the difficult problem of phage taxonomy.

Lysogenic Conversion. The presence of a prophage in a lysogenic culture affects bacterial properties in a variety of ways that are not always predictable. Besides the propensity toward spontaneous and induced phage production, and the prophage specific immunity, several characters associated with the lysogenic state have been described. Only two examples will be mentioned here.

Burnet (1934) had noted a constant association of certain phages with specific bacterial types in *Salmonella*. In a well-known example, lysogenization of certain bacterial species of group E1 (antigens 3, 10) with phages carried by group E2 converts the recipient bacteria into strains of group E2

(antigens 3, 15) (Uetake et al, 1955). In some instances of this type, the converted strains lose the ability to adsorb the converting phage, presumably in consequence of the antigenic change Similarly, lysogenization of appropriate strains of nontoxigenic *Corynebacterium* with phages derived from toxigenic strains produces toxigenic strains (Freeman, 1951, Groman, 1955).

In such instances there is a constant association between a specific phage and a specific bacterial property. In several instances it is currently being found that the converted trait can be recognized not only in lysogenic bacteria but also soon after infection in bacteria that are going to lyse (Barksdale, Luria, Uetake, Zinder, personal communications)

Mutations Affecting Lysogeny. Temperate phages are subject to the same classes of mutation seen in virulent phages, and the same genetic methods of analysis are applicable in both cases (Jacob and Wollman, 1955, Kaiser, 1955). In addition, there are certain types of mutation that can be recognized only in temperate phages

One class of mutations (v mutations) confers on the phage the ability to overcome the immunity to superinfection exhibited by bacteria carrying the original phage In one example of this kind, the development of the carried phage is induced by the superinfection, and a mixed yield of virulent and temperate phages results (Jacob and Wollman, 1953).

Mutations suppressing or diminishing the ability to lysogenize (c mutations) have been studied rather thoroughly in coliphage lambda (Kaiser, 1957) and in *Salmonella* phage P22 (Levine, M, 1957) with rather similar findings These mutations occur in a small region of the phage chromosome that can be subdivided into three distinct functional segments, one of which, in lambda, is also the site of v mutations. It appears that the region subject to c and v mutations overlaps with the region determining immunity patterns, which, in turn, is the region controlling the site of prophage localization in the bacterium (Kaiser; see Jacob and Wollman, 1957). This confirms that ability to lysogenize is indeed tied up with mechanisms of immunity production and prophage attachment to the bacterial chromosome and illustrates once more the genetic analysis of complex functions.

Mutations toward virulence (c mutations) are common and occur at multiple chromosome sites which form clusters in the phage chromosome. The reverse mutations are rare and all but unknown. According to the hypothesis of Streisinger and Franklin (1956), this tends to justify the view already expressed that temperateness in phages is a "positive" character and that virulence corresponds to a loss of function.

Defective Prophage. Another class of mutations peculiar to temperate phages gives rise to so-called *defective lysogenic bacteria* These are obtained most conveniently by exposing typical lysogenic cultures to large doses of ultraviolet light and selecting survivors that retain the characteristic immunity to phage but otherwise are apparently nonlysogenic If the original culture was inducible, the defective culture may retain this property, that is, it may lyse after the characteristic latent period following exposure to ultraviolet light, but few or no phage particles are produced.

Each defect can be traced to a definite locus on the phage chromosome and to an interruption of phage growth at some stage in the lytic cycle. In some defective cultures vegetative reproduction occurs following induction (as tested by superinfection experiments using genetically marked phages), and phage specific antigens are formed, but no morphologically intact phage particles (Jacob and Wollman, 1956) In such instances the defect resembles that observed in cultures of T2 inhibited by proflavine (DeMars, 1955)

In general, phage production is normal if the defective culture is induced (to lift the immunity) and superinfected with normal phage. Under these conditions genetic and physiologic contributions from the defective prophage can be recognized (Jacob and Wollman, 1956; Jacob et al, 1957) In certain instances, the mixed infection gives rise to morphologically intact particles carrying the genetic defect. Such particles are noninfective except that they can again establish defective lysogeny with low frequency (Appleyard, 1956). In mixed infections of this kind, the defective function is evidently made good by the nondefective phage, a phenomenon made use of in the test of functional complementarity (Benzer, 1955)

Defective lysogeny is instructive in several respects. The responsible mutations affect primarily the lytic cycle of phage growth but would pass unnoticed in virulent phages because they would be lethal. Just as a virulent phage may be regarded as one that has lost the power to lysogenize, a defective prophage corresponds to a phage that has lost the ability to complete the lytic cycle of phage growth. It functions very nearly as an ordinary determinant of bacterial heredity. Most important, mutations producing defective prophages offer a means of analyzing phage growth and morphogenesis by the usual methods of chemical genetics (Jacob et al., 1957).

Mixed Inheritance. Since the general characteristics of lysogeny began to be appreciated, the idea of genetic homology between bacterium and phage has seemed to be increasingly appropriate (Lwoff, 1953, Bertani, 1953). In its origins, this idea merely reflected the fusion of phage inheritance and bacterial inheritance seen in lysogenic bacteria. Since 1953, however, the idea has already acquired a long history of more or less useful applications (Fraser, 1957, Stent, 1958). Finally, a defective prophage has been found which seems to unite in its structure mutational markers of authentic bacterial and viral origins and can give rise to bastard phage particles. This development is described below in connection with the subject of transduction.

INFERENCES IV

The lysogenic character has been aptly described as a hereditary property of a bacterium that can be acquired by infection with a virus. Once established, it differs from other bacterial markers only by virtue of its special lethal potential, which is the price paid by the bacterium for remarkable gains in genetic versatility.

The lysogenic bacterium harbors a well-defined prophage. The main biologic affinities of the prophage are well illustrated by two classes of mutation to which it is subject. A temperate phage can lose the power to lysogenize, becoming "pure virus." A temperate phage can also lose its viral potential, becoming a defective prophage. A defective prophage is a gene cluster in a bacterium. Only a sophisticated virologist can trace its origin in a phage.

OTHER CONSEQUENCES OF INFECTION

Mixed Infection. Genetic recombination, phenotypic mixing, multiplicity reactivation and cross reactivation can be interpreted, in broad terms, as intelligible genetic interactions observed in mixed infections. These interactions occur only between related phages and have been described already. Some less intelligible consequences of mixed infection will be mentioned here.

When a bacterium is infected with two unrelated phages (differing morphologically or possessing no common antigens), one or the other, but seldom or never both, produce offspring. This phenomenon is called *mutual exclusion between unrelated phages*, and has received no satisfactory explanation (Weigle and Delbruck, 1951). It is clearly due to genetic differences between the phages and should not be confused with the immunity characterizing the lysogenic state, which obeys very different rules. However, lysogenic bacteria are refractory to infection by certain heterologous phages, and this phenomenon could be related to mutual exclusion, though it seems to be much less general (Lederberg 1957).

If mixed infection is brought about by distantly related phages, such as T2 and T4, one of the pair tends to be excluded, completely in some bacteria and partially in others. This effect is also clearly due to genetic differences between the phages. Among the progeny of crosses between T2 and T4, Streisinger and Weigle (1956) found that the ability of T4-like particles to exclude T2 is correlated with other characteristics, including high DNA-glucose content, that do not segregate as do the usual genetic markers. This type of exclusion may be characterized as *partial exclusion between distantly related phages*.

In simultaneous infection with several identical phages there is also probably a small amount of exclusion. This can be shown by mixed infection with 10 to 20 particles of T2 and 1 or 2 particles of an r mutant of T2

per bacterium Under these conditions a certain proportion of the mixedly infected cells will not yield any *r* phages, an effect which probably cannot be ascribed to the r marker itself. Dulbecco (1949a) interpreted this phenomenon as a limitation to the number (about 10 in his experiments) of particles that can participate in phage growth in one cell. However, if it is assumed that not all particles inject simultaneously, this limited participation could be only another expression of the well-defined phenomenon to be described next.

Partial exclusion of superinfecting phage is seen when bacteria are infected first with one and then with another of two very closely related phages It does not depend (except for convenience of demonstration) on genetic differences between the two phages The resistance to superinfection is demonstrable within 1 minute after infection (Dulbecco, 1949b) but probably does not become maximal for several minutes Resistance to superinfection by T2 is accompanied by resistance to lysis from without (Visconti, 1953), by reduced efficiency of injection (Hershey et al, 1954), by cyclical changes in cell premeability (Puck and Lee, 1955), and by the ability of the cells to decompose the DNA of superinfecting phage particles (Graham, 1953) The exclusion is partial in respect to all these phenomena, and some of the superinfecting particles always succeed in infecting (Visconti, 1953). All of the effects mentioned can be explained by assuming some change in the cell surface that interferes with normal injection Resistance to superinfection of this type has been studied mainly with *T2* and is less marked or absent with some other phages

Host-Controlled Variation The terms "host-controlled variation" or "host-induced modification" refer to the fact that the host-range property of phage particles is sometimes

not affect injection. It presumably reflects an alteration of some internal component of the phage particle, but of what kind is not known The phenomenon differs from typical mutation in several respects, notably by the fact that all the particles issuing from the modifying host are usually modified, independently of the state of the infecting particle. For this reason host-controlled variation is often called phenotypic or, more accurately, nonheritable Further details will be found in reviews by Luria (1953) and Adams (1958).

Transduction is a remarkable process by which factors from a genetically marked donor bacterium may be carried into an acceptor bacterium by a phage particle, modifying the bacterial inheritance (Zinder, 1955). The phenomenon has been used extensively for genetic analysis of bacteria (reviewed by Hartman, 1957) We present here only the main facts that seem to bear on the biology of phage

To begin with, one must distinguish between two kinds of transduction, seen with different phages "Typical transduction," observed with several phages, shows the following characteristics (Zinder, 1955):

1. The material carried by the phage consists of a fragment of a bacterial chromosome which effectively substitutes for a corresponding region of the chromosome of the acceptor bacterium. The fragment usually accommodates only a single bacterial marker but may include 2 or more linked markers (Lennox 1955, Jacob, 1955, Demerec and Demerec, 1956)

2 The effective material is derived exclusively from the bacterium in which the vector phage particle was produced; it shows no relation to the germ line of the phage The acceptor bacterium need not become lysogenic. Both facts distinguish typical transduction from lysogenic conversion

3 All bacterial characters that have been tested are transducible Typical transduction furnishes no clue in this respect to the presumed site occupied by the prophage in lysogenic bacteria

4. Only a small fraction (10^{-6}) of particles in a given preparation appears to be competent in the transduction of a given marker It is not known whether these particles are infective in the usual sense (Zinder, personal communication).

5. Phage particles heavily irradiated with ultraviolet light or subjected to decay of incorporated radiophosphorus, treatments effective in destroying infectivity of phage, do not destroy the transducing power of the particles (Garen and Zinder, 1955).

These facts are consistent with the idea that when a phage particle matures, bits of chromosomal material of bacterial origin can be included in it by accident According to this interpretation, transduction is only a special case of transformation of bacterial characters by DNA (Lederberg, 1955). This view suggests either that some fraction of the DNA in a phage particle is superfluous to the infective property or, if transducing particles are not infective, that the maturation process has imperfect discriminatory power toward DNA. Indeed, the discovery of transductions of lysogeny (Jacob, 1955, Lennox, 1955) suggested that there might be room in a single phage particle for intact chromosomes derived from 2 or 3 phage lines and fragments from the host as well Various reservations to this conclusion were necessary at the time, and the study of transduction by phage lambda has since shown that transducing phage particles in that system are in fact noninfective Therefore, the significance of typical transduction for questions about phage structure is not clear

Transduction observed in *E. coli* K12 by phage lambda (lambda transduction) differs from that described above in several ways The differences should be ascribed to the phage, not to the bacterium, because transduction in K12 by phage P1 is typical (Lennox 1955) Lambda transduction shows the following distinctive features (Morse et al., 1956).

1. Transducing phages are obtained in the first instance by induction of lysogenic bacteria with ultraviolet light, never by infecting sensitive bacteria

2 The cells modified by transduction always carry prophages

3 Only the galactose fermentation markers adjacent to the prophage site are transducible

4 Most of the cells modified by transduction do not arise by genetic substitution as in typical transduction but by addition of genetic material, they persistently throw off daughter cells of the acceptor type with low frequency These genetically unstable cell lines are called heterogenotes

5 Induction of phage production in heterogenotes yields phage, called HFT, transducing the galactose character with very high frequency.

The availability of HFT phage permits experimentation with a precision previously unattainable in transduction experiments Owing to this fact, the following additional information could be obtained (Weigle, 1957; Campbell, 1957, Arber et al, 1957)

6. HFT phage consists of a mixture of normal phage particles and defective particles in approximately equal numbers. The normal particles are nontransducing The defective particles adsorb to bacteria but mostly kill and lyse them without multiplying However, a few of them produce new heterogenotes, but these prove to be defective lysogens High-frequency transduction, yielding actively lysogenic heterogenotes, is produced only by mixed infection with normal particles and defective particles, the latter performing the actual transduction

7 Therefore, the actively lysogenic heterogenote is a doubly lysogenic bacterium carrying a normal prophage associated with the intact bacterial chromosome of the acceptor genotype, and a defective prophage, associated with a bacterial chromosome fragment of the donor genotype On induction, both prophages initiate phage production, the defective phage being helped out by the normal one, and a mixed yield of defective particles and normal particles results

8 When the defective phage multiplies vegetatively, which it can do only in bacteria also infected with normal phage, the bacterial chromosome fragment associated with the defective prophage multiplies too Thus, whether associated with prophage or vegetative phage, the bacterial chromosome fragment behaves like part of the defective phage chromosome

9 In crosses between genetically marked defective phage and normal phage, certain characters can be separated from the defect and recovered in live phage particles However, a segment of the defective chromosome corresponding to the host range and adjacent markers cannot be recovered in this way, nor does it function in the crosses to produce phenotypic mixing Therefore, the defect lies in the region controlling host range and may correspond to a deletion of genetic material

All this suggests that transducing phages, in the lambda system, arise not by inclusion of extra material in the phage particle but by substitution of bacterial markers for phage markers, yielding a chromosome of mixed

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teriophage reproduction. Cold Spring Harbor Symp Quant. Biol *18*, 245-252

Brown, H D, and Kozloff, L M 1957, Morphological localization of the bacteriophage tail enzyme, J Biol Chem *225*, 1-11

Brown, G L., and Martin, A V 1956, Fractionation of the deoxyribonucleic acid of T2r bacteriophage, Nature, London *176*, 971-972

Burnet, F M., 1934, The bacteriophages, Biol Rev. Cambridge *9*, 332-350

Burnet, F M., and McKie M., 1929, Observations in a permanently lysogenic strain of *B enteritidis gaertner*, Australian J Exper Biol & M Sc *6*, 277-284

Campbell, A, 1957, Transduction and segregation in *Escherichia coli* K12 Virology *4*, 366-384

Cheng, P., 1956, Properties of tryptophan-inactivated bacteriophage T4,38, Biochim et biophys acta *22*, 443-451

Cohen, S S, 1949, Growth requirements of bacterial viruses, Bact Rev *13*, 1-24

Delbrück, M, and Stent, G S, 1957, On the mechanism of DNA replication, *in* McElroy and Glass (ed), pp 699-736

DeMars, R I, 1953, Chemical mutagenesis in bacteriophage T2, Nature, London *172*, 964-965

———, 1955, The production of phage-related materials when bacteriophage development is interrupted by proflavine, Virology *1*, 83-99

Demerec, M, and Demerec, Z., 1956, Analysis of linkage relationships in *Salmonella* by transduction techniques, Brookhaven Symposia Biol *8*, 75-84

Doermann, A H., 1952, The intracellular growth of bacteriophages I Liberation of intracellular bacteriophage T4 by premature lysis with another phage or with cyanide J Gen Physiol *35*, 645-656

———, 1953, The vegetative state in the life cycle of bacteriophage evidence for its occurrence and its genetic characterization, Cold Spring Harbor Symp Quant Biol *18*, 3-11

Doermann, A H, Chase, M, and Stahl, F W., 1955, Genetic recombination and replication in bacteriophage, J Cell & Comp Physiol *45* (Suppl 2), 51-74

Doermann, A H, and Dissosway, C F-R, 1949, Intracellular growth and genetics of bacteriophage, Carnegie Inst Washington Yrbk *48*, 170-176

Dulbecco, R, 1949a, The number of particles of bacteriophage T2 that can participate in intracellular growth, Genetics *34*, 126-132

———, 1949b, Mutual exclusion between related phages, J Bacteriol *63*, 209-217

———, 1955, Photoreactivation, *in* Hollaender, A. (ed) Radiation Biology, vol 2, pp 455-486, New York, McGraw-Hill

Ellis, E L., and Delbruck, M, 1939, The growth of bacteriophage, J Gen Physiol *22*, 365-384

Fraser, D, Mahler, H, Shug, A, and Thomas, C., Jr., 1957, The infection of subcellular *Escherichia coli* strain B with free DNA from T2 bacteriophage, Proc Nat Acad Sc *43*, 939-947

Fraser, H K, 1957, Host range mutants and semitemperate mutants of bacteriophage T3, Virology *3*, 527-553

Freeman, V J., 1951, Studies on the virulence of bacteriophage-infected strains of *Corynebacterium diphtheriae*, J Bacteriol *61*, 407-414

Garen, A, and Zinder, N D, 1955, Radiological evidence for partial genetic homology between bacteriophage and host bacteria, Virology *1*, 347-376

Goodgal, S H., Rupert, C S, and Herriott, R M., 1957, Photoreactivation of *Hemophilus influenzae* transforming factor for streptomycin resistance by an extract of *Escherichia coli* B *in* McElroy and Glass (ed), pp 341-343

Gots, J S, 1958, Chapter 15 in Adams (1959)

Graham, A F., 1953, The fate of the infecting phage particle, Ann Inst Pasteur *84*, 90-98

Groman, N B., 1955, Evidence for the active role of bacteriophage in the conversion of nontoxigenic *Corynebacterium diphtheriae* to toxin production, J Bacteriol *69*, 9-15

Hartman, P E., 1957, Transduction a comparative review, *in* McElroy and Glass (ed), pp 408-462

d'Herelle, F., 1917, Sur un microbe invisible antagoniste des bacilles dysenteriques, Compt rend Acad Sc *165*, 373-375

———, 1926, The Bacteriophage and its Behavior, Baltimore, Williams & Wilkins

Herriott, R M., 1951, Nucleic acid-free T2 virus "ghosts" with specific biological action, J Bacteriol *61*, 752-754

Hershey, A D., 1946, Spontaneous mutations in bacterial viruses, Cold Spring Harbor Symp Quant Biol *11*, 67-76

———, 1952, Reproduction of bacteriophage Internat Rev Cytol *1*, 119-134

———, 1953, Inheritance in bacteriophage *in* Advances in Genetics, vol 5, pp 89-106, New York, Acad Press

———, 1956, The organization of genetic material in bacteriophage T2, Brookhaven Symposia Biol *8*, 6-14

———, 1957a, Bacteriophages as genetic and biochemical systems *in* Advances Virus Research, vol 4, pp 25-61, New York, Acad Press

———, 1957b, Some minor components of bacteriophage T2 particles, Virology *4*, 237-264

Hershey, A D., and Burgi, E., 1956, Genetic significance of the transfer of nucleic acid from parental to offspring phage, Cold Spring Harbor Symp Quant Biol *21*, 91-101

Hershey, A D, and Chase, M., 1951, Genetic recombination and heterozygosis in bacteriophage, Cold Spring Harbor Symp Quant Biol *16*, 471-479

———, 1952, Independent functions of viral protein and nucleic acid in growth of bacteriophage, J Gen Physiol *36*, 39-56

Hershey, A D, Garen, A., Fraser, D K., and Hudis, J D, 1954, Growth and inheritance in bacteriophage, Carnegie Inst Washington Yrbk. *53*, 210-225

Hershey, A D, and Melechen, N E., 1957, Synthesis of phage precursor nucleic acid in the presence of chloramphenicol, Virology *3*, 207-236

origin. In any case, the process is clearly not random, the foreign material included in the phage particle is derived from the region of the bacterial chromosome adjacent to the prophage site, and its inclusion gives rise to a specific hereditary defect in phage function

INFERENCES V

In lysogeny one sees an integration of phage material and bacterial material in which the integrated unit behaves as a nearly normal *bacterial chromosome In typical transduction*, one sees a mechanism for the transfer of genetic material from one bacterial chromosome into another, in which the phage plays a purely mechanical role. In typical transduction, unlike lysogeny, there is no fusion of phage inheritance and bacterial inheritance. Transduction by phage lambda is neither transduction nor lysogeny, as understood previously. As in lysogeny, one sees an integration of genetic material from phage and bacterium, but the unit of integration is not a bacterial chromosome or a phage chromosome but something having resemblances to both On the one hand, aided by a normal phage chromosome it multiplies vegetatively and fits into a phage particle to form a characteristic biologic unit possessing many phage functions but lacking others. On the other hand, it can be perpetuated in unstable association with a normal bacterial chromosome, where it performs some bacterial functions and some prophage functions, resembling a supernumerary chromosome fragment of mixed origin.

The details of these unexpected phenomena are relevant to an eventual generalization that might read something like this: the modalities by which chromosomes express their presumed basic attributes are indefinitely various because history has presented genetic materials with innumerable opportunities. *It follows from this generalization that every genuinely new method of looking at living things must reveal unexpected phenomena, and it is only by cultivating his astonishment that the observer can hope to achieve a grasp of the presumed basic attributes. Perhaps the first* main point to emerge from the study of temperate phages is that phage heredity and bacterial heredity are interwoven quite as closely as are phage and bacterial functions; then the second main point would be that the interweaving is not inextricable.

The role of DNA as the material basis of heredity in phages and bacteria, though now factually clear, does not yet lend itself to generalization owing to the growing evidence that other viruses, and perhaps phages too at some time in their life cycle, can dispense with DNA The existence of "DNA viruses" and "RNA viruses," for the present, merely raises questions that escape us Do, for instance, viruses and their hosts generally form com*mon reservoirs of genetic material or is this* a corner of DNA history to which only certain phages and bacteria have access?

REFERENCES

Adams, M H., 1958, Bacteriophages, New York, Interscience

Anderson, T F, 1953, The morphology and osmotic properties of bacteriophage systems, Cold Spring Harbor Symp Quant Biol *18*, 197-203

Anderson, T F, and Doermann, A H, 1952, The intracellular growth of bacteriophages II The *growth of T3 studied by sonic disintegration* and by T6-cyanide lysis of infected cells, J. Gen Physiol *35*, 657-667

Appleyard, [illegible] K, 1956, The transfer of defective lambda lysogeny between strains of *Escherichia coli*, J Gen Microbiol *14*, 573-582

Arber, W., Kellenberger, G, and Weigle, J, 1957, La defectuosite du phage lambda transducteur. Schweiz Ztschr allg Path *20*, 659-665

Benzer, S, 1952, Resistance to ultraviolet light as an index to the reproduction of bacteriophage, J Bacteriol *63*, 59-72

———, 1955, Fine structure of a genetic region in bacteriophage, Proc Nat Acad Sc *41*, 344-354

———, 1957, The elementary units of heredity, *in* McElroy and Glass (ed), pp 70-93

Bertani, G, 1951, Studies on lysogenesis I The mode of phage liberation by lysogenic *Escherichia coli* J Bacteriol *62*, 293-300

———, 1953, Lysogenic versus lytic cycle of phage multiplication, Cold Spring Harbor Symp Quant Biol *18*, 65-70

———, 1956, The role of phage in bacterial genetics, Brookhaven Symposia Biol *8*, 50-56

———, 1958, Lysogeny *in* Advances in Virus Research, vol 5, pp 151-193. New York, Acad Press

Bertani, L E, 1957, The effect of inhibition of protein synthesis on the establishment of lysogeny, Virology *4*, 53-71.

Beumer, J., Dirkx, J, and Beumer-Jochmans, M P., 1957, Role of cations in phage adsorption to sensitive bacteria, Nature, London *180*, 83-85

Bowen, G H., 1953, Studies of ultraviolet irradiation phenomena—an approach to the problems of bac-

exponential rate of phage reproduction Cold Spring Harbor Symp Biol *16*, 463-470

Luria, S E, 1953, Host-induced modifications of viruses, Cold Spring Harbor Symp Quant Biol *18*, 237-244

Luria, S E, and Anderson, T F, 1942, The indentification and characterization of bacteriophages with the electron microscope, Proc Nat Acad Sc *28*, 127-130

Luria, S E, and Delbruck, M, 1943, Mutations of bacteria from virus sensitivity to virus resistance, Genetics *28*, 491-511

Luria, S E, and Dulbecco, R, 1949, Genetic recombinations leading to production of active bacteriophage from ultraviolet inactivated bacteriophage particles, Genetics *34*, 93-122

Luria, S E, and Latarjet, R, 1947, Ultraviolet irradiation of bacteriophage during intracellular growth, J Bacteriol *53*, 149-163

Luria, S E, and Steiner, D L, 1954, The role of calcium in the penetration of bacteriophage T5 into its host, J Bacteriol *67*, 635-639

Luria, S E, Williams, R C, and Backus, R C, 1951, Electron micrographic counts of bacteriophage particles, J Bacteriol *61*, 179-188

Lwoff, A, 1953, Lysogeny, Bact Rev *17*, 269-337

Lwoff, A., and Gutmann, A, 1950, Recherches sur un *Bacillus megatherium* lysogene, Ann Inst Pasteur *78*, 711-739

Maaløe, O, and Symonds, N, 1953, Radioactive sulfur tracer studies on the reproduction of T4 bacteriophage, J Bacteriol *65*, 177-182

McElroy, W D, and Glass, B (ed), 1957, The Chemical Basis of Heredity, Baltimore Johns Hopkins Press

Miller, E M, and Goebel W F, 1954, Nature of prophage in lysogenic bacteria, J Exper Med *100*, 525-540

Morse, M L, Lederberg, E, and Lederberg, J, 1956, Transductional heterogenotes in *Escherichia coli*, Genetics *41*, 758-779

Novick, A, and Szilard, L, 1951, Virus strains of identical phenotype but different genotype, Science *113* 34-35

Puck, T T, and Lee, H H, 1955, Mechanism of cell wall penetration by viruses II Demonstration of cyclic permeability change accompanying virus infection of *Escherichia coli* B cells J Exper Med *101*, 151-175

Putnam, F W, and Kozloff, L M, 1950, Biochemical studies of virus reproduction IV The fate of the infecting virus particle, J Biol Chem *182*, 243-250

Sato, G H, 1956, Activation of bacteriophage by urea, Science *123*, 891 892

Sinsheimer, R L, 1956, The glucose content of the deoxyribonucleic acids of certain bacteriophages, Proc Nat Acad Sc *42*, 502-504

———, 1957, Some properties of bacteriophage ΦX174, Fed Proc *16*, 250

Spizizen, J, 1957, Infection of protoplasts by disrupted T2 virus, Proc Nat Acad Sc *43*, 694-701

Stahl, F W, 1956, The effects of decay of incorporated radioactive phosphorus in the genome of bacteriophage T4, Virology *2*, 206-234

Stent, G S, 1953, Cross reactivation of genetic loci of T2 bacteriophage after decay of incorporated radioactive phosphorus, Proc Nat Acad Sc *39*, 1234-1241.

———, 1958, Mating in the reproduction of bacterial viruses *in* Advances in Virus Research, vol 5, pp 95-149 New York, Acad Press

Stent, G S, and Fuerst, C R, 1955, Inactivation of bacteriophages by decay of incorporated radioactive phosphorus, J Gen Physiol *38*, 441-458

———, 1956, Decay of incorporated radioactive phosphorus during development of a temperate bacteriophage, Virology *2*, 737-752

Stent, G S, Fuerst, C R, and Jacob, F, 1957, Inactivation d'un prophage par la désintégration du radiophosphore, Compt rend Acad Sc *244*, 1840-1842.

Stent, G S, and Jerne, N K, 1955, The distribution of parental phosphorus atoms among bacteriophage progeny, Proc Nat Acad Sc *41*, 704-709

Streisinger, G, 1956a, The genetic control of host range and serological specificity in bacteriophages T2 and T4, Virology *2*, 377-387

———, 1956b, Phenotypic mixing of host range and serological specificities in bacteriophages T2 and T4, Virology *2*, 388-398

Streisinger, G, and Franklin, N C, 1956, Mutation and recombination at the host range genetic region of phage T2, Cold Spring Harbor Symp Quant Biol *21*, 103-109

Streisinger, G, and Weigle, J, 1956, Properties of bacteriophages T2 and T4 with unusual inheritance, Proc Nat Acad Sc *42*, 504-510

Taylor, N W, Epstein, H T, and Lauffer, M A, 1955, The particle weight, hydration and shape of the T2 bacteriophage of *Escherichia coli*, J Am Chem Soc *77*, 1270-1273

Tessman, I, Tessman, E S, and Stent, G S, 1957, The relative radiosensitivity of bacteriophages S13 and T2, Virology *4*, 209-215

Tolmach, L J, 1957, Attachment and penetration by viruses *in* Advances in Virus Research, vol 4, pp 63-110, New York, Acad Press

Tomizawa, J, 1958, Sensitivity of phage precursor nucleic acid, synthesized in the presence of chloramphenicol, to ultraviolet irradiation Virology, in press

Uetake, H, Nakagawa, T, and Akiba, T, 1955, The relationship of bacteriophage to antigenic changes in salmonellas of group E, J Bacteriol *69*, 571-579

Vidaver, G A, and Kozloff, L M, 1957, The rate of synthesis of deoxyribonucleic acid in *Escherichia coli* B infected with T2 bacteriophage, J Biol Chem *225*, 335-347.

Visconti, N, 1953, Resistance to lysis from without in bacteria infected with T2 bacteriophage, J Bacteriol *66*, 247-253

Visconti, N, and Delbruck, M, 1953, The mechanism of genetic recombination in phage, Genetics *38*, 5-33

Visconti, N, and Garen, A, 1953, Unity of the vegetative pool in phage-infected bacteria, Proc Nat Acad Sc. *39*, 620-627

Hershey, A D, and Rotman, R, 1948, Linkage among genes controlling inhibition of lysis in a bacterial virus, Proc Nat Acad Sc *34*, 89-96
———, 1949, Genetic recombination between host range and plaque type mutants of bacteriophage in single bacterial cells, Genetics *34*, 44-71
Hook, A E, Beard, D, Taylor, A R, Sharp, D G, and Beard, J W, 1946, Isolation and characterization of the T2 bacteriophage of *Escherichia coli*, J Biol Chem *165*, 241-258
Hotchin, J E, 1954, The purification and electron microscopical examination of the structure of staphylococcal bacteriophage K, J Gen Microbiol *10*, 250-260
Jacob, F, 1954, Mutation d'un bactériophage induite per l'irradiation des seules bactéries-hôtes avant l'infection, Compt rend. Acad Sc *233*, 732-734
———, 1955, Transduction of lysogeny in *Escherichia coli*, *Virology 1*, 207-220
Jacob, F, Fuerst, C R, and Wollman, E L, 1957, Recherches sur les bactéries lysogènes défectives II Les types physiologiques liés aux mutations du prophage Ann Inst Pasteur *93*, 724-753
Jacob, F, and Wollman, E L, 1953, Induction of phage development in lysogenic bacteria, Cold Spring Harbor Symp Quant Biol *18*, 101-210
———, 1955, Étude génétique d'un bactériophage tempéré *d'Escherichia coli* III Effet du rayonnement ultraviolet sur la recombination génétique Ann Inst Pasteur *88*, 724-749
———, 1956, Recherches sur les bacteries lysogenes defectives I Determinisme genetique de la morphogenèse chez un bacteriophage tempere Ann Inst Pasteur *90*, 282-302
———, 1957, Genetic aspects of lysogeny, *in* McElroy and Glass (ed), pp 468-499
Jerne, N K, 1956, The presence in normal serum of specific antibody against bacteriophage T4 and its increase during the earliest stages of immunization, J Immunol *76*, 209-216
Jerne, N K, and Avegno, P, 1956, The development of the phage-inactivating properties of serum during the cause of specific immunization of an animal reversible and irreversible inactivation, J Immunol *76*, 200-208
Jesaitis, M A, 1957, The nucleic acids of T2, T4, and T6 bacteriophages, J Exper Med *106*, 233-246
Jesaitis, M A, and Goebel W F, 1955, Lysis of T4 phage by the specific lipocarbohydrate of phase II *Shigella sonnei*, J Exper Med *102*, 733-752
Kaiser, A D, 1955, A genetic study of the temperate coliphage lambda, Virology *1*, 424-443
———, 1957, Mutations in a temperate bacteriophage affecting its ability to lysogenize *Escherichia coli*, Virology *3*, 42-61
Kellenberger, E, and Arber, W, 1955, Die struktur des Schwanzes der Phagen T2 und T4 und der Mechanismus der irreversiblen Adsorption, Ztschr Naturforsch. *10b*, 698-704
———, 1957, Electron microscopical studies of phage multiplication I A method for quantitative analysis of particle suspensions Virology *3*, 245-255
Koch, G, and Weidel, W, 1956, Abspaltung chemischer Komponenten der Coli-Membran durch daran adsorbierte Phagen, Ztschr Naturforsch *11b*, 345-353.
Kozloff, L. M., and Lute, M., 1957, Viral invasion II The role of zinc in bacteriophage invasion J Biol Chem *228*, 529-536
Kozloff, L M, Lute, M, and Henderson, K, 1957, Viral invasion I Rupture of thiolester bends in the bacteriophage tail. J Biol Chem *228*, 511-528
Krieg, D R, 1957, A study of gene action in ultraviolet-irradiated bacteriophage Dissertation, University of Rochester
Lanni, F, and Lanni, Y T, 1953, Antigenic structure of bacteriophage, Cold Spring Harbor Symp Quant Biol *18*, 159-168
Latarjet, R, 1949, Mutation induite chez un virus par irradiation ultraviolet de cellules infectées, Compt rend Acad Sc *228*, 1354-1356
Lederberg, E M, and Lederberg, J, 1953, Genetic studies of lysogenicity in *Escherichia coli*, Genetics *38*, 51-64
Lederberg, J, 1955, Recombination mechanisms in bacteria, J Cell & Comp Physiol *45* (Suppl 2), 75-107
Lederberg, J, and Tatum, E L, 1946, Novel genotypes in mixed cultures of biochemical mutants of bacteria, Cold Spring Harbor Symp Quant Biol *11*, 113-114
Lederberg, S, 1957, Suppression of the multiplication of heterologous bacteriophages in lysogenic bacteria, Virology *3*, 496-513
Lennox, E S, 1955, Transduction of linked genetic characters of the host by bacteriophage P1, Virology *1*, 190-206
Lennox, E S, Luria, S E, and Benzer, S, 1954, On the mechanism of photoreactivation of ultraviolet-inactivated bacteriophage, Biochem et biophys acta *15*, 471-474
Levine, L, VanVanakis, H, and Barlow, J, 1957, Personal communication
Levine, M, 1957, Mutations in the temperate phage P22 and lysogeny in *Salmonella*, Virology *3*, 22-41
Levinthal, C, 1954, Recombination in phage T2 its relationship to heterozygosis and growth, Genetics *39*, 169-184
———, 1956, The mechanism of DNA replication and genetic recombination in phage, Proc Nat Acad Sc *42*, 394-404
Lieb M 1953, The establishment of lysogenicity in *Escherichia coli*, J Bacteriol *65*, 642-651
Litman, R M, and Pardee, A B, 1956, Production of bacteriophage mutants by a disturbance of deoxyribonucleic acid metabolism, Nature, London *178*, 529-531
Luria, S E, 1945, Mutations of bacterial viruses affecting their host range, Genetics *30*, 84-99
———, 1947, Reactivation of irradiated bacteriophage by transfer of self-reproducing units, Proc Nat Acad Sc *33*, 253-264
———, 1950, Bacteriophage an essay on virus reproduction, Science *111*, 507-511
———, 1951, The frequency distribution of spontaneous bacteriophage mutants as evidence for the

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8

Chick-Embryo Technics

INTRODUCTION

For centuries the developing chick embryo has attracted a wide range of interest in medical and biologic research Since 1931 much progress has been made in its adaptation for the propagation of infectious agents and for the study of the diseases they produce. The embryonated hen's egg has been found to be particularly well suited for many of the viruses and most of the rickettsiae known to be pathogenic for man and animals Under proper conditions experimental infections provoke typical disease reactions in the embryo Therefore, it is one of the most useful laboratory animals for the study of the pathogenesis of infectious diseases and the analysis of many factors related to immunity.

Isolation and identification of the causative agent by inoculation of chick embryos is now standard procedure for many viral and rickettsial diseases Large quantity production from infected chick embryos has furthered much knowledge regarding the physical, chemical and many other basic properties of several viruses Assembly line procedures have been developed for the preparation of vaccines from this source Important diagnostic antigens are obtained by the chick embryo method The biologic assay of many therapeutic agents is carried out by this means

HISTORY

The general use of the chick embryo for the study and the propagation of infectious agents began to develop after Woodruff and Goodpasture (1931) described infection of the chorio-allantoic membrane with fowlpox virus. Later in the same year they reported with Buddingh (1931) that the viruses of vaccinia and herpes simplex also could be propagated by this method A successful smallpox vaccine prepared from infected chorio-allantoic membranes was reported almost simultaneously by Goodpasture and Buddingh (1933) and by Stevenson and Butler (1933) from England. These experiences prompted Goodpasture (1933) to emphasize the potentialities of the chick-embryo method for most of the purposes to which it has been adapted since then He and his associates explored many of its uses for the study of viral and bacterial infections and provided the basis for many of its practical applications

Beginning in 1933, Burnet and his collaborators contributed much to the advance of the new technic Their efforts were particularly significant in devising means for utilizing the chick embryo for quantitative studies The lethal effect of fowl plague and Newcastle disease viruses on the embryo was

wider surface area This made the pock-counting technic feasible by means of which quantitative studies with vaccinia, herpes simplex, ectromelia and their specific antibodies were undertaken

Watanabe, I, 1957, The effect of ultraviolet light on the production of bacterial virus protein, J Gen Physiol. *40*, 521-531

Watanabe, I, Stent, G S., and Schachman, H K, 1954, On the state of the parental phosphorus during reproduction of bacteriophage T2, Biochem et biophys acta *15*, 38-49

Watson, J D, 1952, The properties of X-ray inactivated bacteriophage II Inactivation by indirect effects J. Bacteriol *63*, 473-485

Weidel, W, and Kellenberger, E, 1955, The E. coli B receptor for the phage T5 II Electron microscopic studies Biochem et biophys. acta *17*, 1-9

Weigle, J, 1953, Induction of mutations in a bacterial virus, Proc Nat Acad Sc *39*, 628-636

———, 1957, Transduction by coliphage lambda of the galactose marker, Virology *4*, 14-25.

Weigle, J, and Delbruck, M, 1951, Mutual exclusion between an infecting phage and a carried phage, J. Bacteriol *62*, 301-318

Weigle, J., and Dulbecco, R., 1953, Induction of mutations in bacteriophage T3 by ultraviolet light, Experientia *9*, 372-373.

Williams, R C, and Fraser, D, 1953, Morphology of the seven T-bacteriophages, J Bacteriol *66*, 458-464

———, 1956, Structural and functional differentiation in T2 bacteriophage, Virology *2*, 289-307.

Zinder, N. D, 1955, Bacterial transduction, J. Cell & Comp Physiol *45* (Suppl. *2*), 23-49.

munization imposed by World War II greatly accelerated development of the chick-embryo method for the production of yellow fever, typhus, influenza and other vaccines

Before 1931, several experiments presaged the eventual use of the chick embryo for these purposes. Thus, Rous and Murphy (1911) propagated and studied the effect of the agent of chicken sarcoma in the developing egg Jouan and Staub (1920) propagated fowl-pest virus by this means Gay and Thompson (1929) described an increase of vaccinia virus introduced into the yolk sac of chick embryos Goodpasture (1938) quotes Levaditi (1906) to indicate that Borrel perhaps was the first to use the developing egg for the study of infection In 1904 Borrel studied spirillosis of fowls after inoculation of fertile and unfertile incubating eggs More detailed accounts of the history of the development of the method are available in Burnet (1936), Goodpasture (1938), Beveridge and Burnet (1946) and Burnet (1953)

GENERAL CONSIDERATIONS

The basic chick-embryo technics are now standard methods The publications of Goodpasture and Buddingh (1935), Burnet (1936), Polk et al (1938) Beveridge and Burnet (1946) and Buddingh (1953) as well as several recent textbooks and manuals can be consulted for descriptions of the essential procedures One of the outstanding features of the method is its versatility in that different routes of inoculation are practicable Thus the chorio-allantoic membrane, the allantoic cavity, the amniotic cavity, the yolk sac, the veins of the membranes and the embryo itself are accessible as sites in which infection can be established Each of these routes of inoculation is best suited to a definite purpose with a particular virus or rickettsia Differences in the performance of details in various procedures have been developed by many investigators to suit the needs of particular research problems Diagnostic laboratories have introduced adaptations which facilitate their work Pharmaceutical establishments interested in large quantities have placed the embryonated egg on the assembly line for the commercial production of vaccines and diagnostic reagents

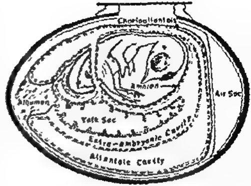

FIG 44 Diagram of embryonated egg of 11 days' incubation showing the important structures involved in the chick-embryo technics

As discussed elsewhere (Buddingh, 1950, 1952a, b) embryonated eggs should not be compared with tubes of nutrient media primarily useful for the propagation of microorganisms Each is a complete self-sustaining laboratory animal which in the short space of 21 days progresses from the fertile egg to the hatched chick This series of changes provides a variety of biologic subtrates to which viruses and rickettsiae are variously adapted The cells of the different tissues and organs of the embryo and of the extra-embryonic structures which support the replication or multiplication of these agents maintain a metabolism in which certain processes predominate during definite stages of incubation These in turn may markedly influence the behavior of the infectious agent and the reaction of the embryonic host During the first 10 days of incubation the metabolic processes from which energy is derived consist mainly of the combustion of carbohydrates, especially glucose, derived from the albumen All types and varieties of cells appear to share in these metabolic processes During this period cellular proliferation and metabolism proceed at their highest rates of activity Extreme and uniform susceptibility with rapid rate of viral proliferation characterizes the 6- to 10-day embryo It is at this stage that the virus-infected embryonated egg perhaps most nearly resembles a test tube culture of bacteria

From the 10th to the 15th day of incubation there is a slowing down of embryonic metabolism. The catabolism of proteins rather

TABLE 7. VIRUSES AND RICKETTSIAE OF HUMAN INFECTIONS PROPAGATED IN THE EMBRYONATED HEN'S EGG *

Disease Agent	Preferred Route of Inoculation	Age of Embryos, Days	Production of Vaccine or Diagnostic Reagent: *Incubation Time*	*Materials Harvested*
Arbor Viruses, African	YS	8-10		
B Virus	CAM	10-12		
Boutonneuse Fever	YS	7	3-5 days	CAM, YS
Colorado Tick Fever	Al	7-8		
Croup Virus (Chanock)	Al	10-11		
Dengue	Al	5		
Epidemic Typhus	YS	7	*5-10 days*	*CAM, YS*
Equine Encephal , East	Al	12	18 hrs	EMB, CAM, Al F
Equine Encephal West	Al	12	24-30 hrs.	EMB, CAM, Al F
Equine Encephal , Ven	YS	7	6-10 days	YS
Herpes Simplex	CAM	10-12		
Influenza A or B	Al	10-12	48 hrs	Al F
Influenza C	Am	10-12		
Influenza D	Al	10-12		
Japanese B Encephal	YS	9	*64 hrs*	*EMB, CAM, Al F*
Louping Ill	YS	9	*3 days*	*EMB, CAM, Al F*
Lymphocytic Chorio	CAM	10-12		
Measles	Am	7-8		
Mumps	Al	8	[illegible] days	Al F
Murine Typhus	YS	7	*5-10 days*	YS
Newcastle Disease	Al	*10-12*	*90 hrs*	*EMB, CAM, Al F*
Poliovirus Type II	Al, YS	7		
Q Fever	YS	7	6-10 days	YS
Rabies	YS	7	8-10 days	YS
Rickettsialpox	YS	7		
Rift Valley Fever	CAM	12		
Rocky Mt Spotted Fever	YS	7	3-4 days	CAM, YS
Scrub Typhus	YS	7		
St Louis Encephalitis	CAM	10 12		
Vaccinia	CAM	10-12	3 days	CAM
Variola	CAM	10-12		
Yellow Fever	YS	7	4 days	EMB

CAM = Chorio-allantoic membrane YS = Yolk sac
Al = Allantoic cavity EMB = Embryo
Am = Amniotic cavity Al F = Allantoic fluid

* Adapted from Cox (1952), Growth of viruses and rickettsiae in the developing chick embryo Ann New York Acad of Sc, 52, 236-247

The experiments of Wilson Smith (1935) marked the beginning of an immense amount of research on the behavior of influenza virus in embryonated eggs This work has been shared by many laboratories in all parts of the world The resulting harvest of informa- [illegible] ference and recombination phenomena of influenza virus has greatly enriched the science of virology.

Zia (1934) in Zinsser's laboratory first cultivated the rickettsiae of murine typhus and of spotted fever in the chorio-allantois Cox (1938) demonstrated the superiority of the yolk sac for the purpose of propagating these agents The demands for mass im-

avian lymphomatosis and possibly ornithosis As pointed out by Cox (1952), endogenous infections have presented no appreciable problem in experimental work or in the production of vaccines

The Inoculum: Fungi, bacteria and viruses may be present as contaminants in the inoculum. Their effect will vary with the age of the embryo and with the route of inoculation Embryonated eggs of less than 11 days incubation are extremely susceptible to contaminants of any type Proteolytic spore-bearing gram-positive bacilli can wreak havoc at any stage. In older embryos the yolk sac more than any other site is vulnerable to the introduction of bacteria The chorio-allantois of embryos 13 days and older will tolerate a surprising variety and number of potentially pathogenic bacteria So-called saprophytic varieties ordinarily do not thrive on the membrane Most contaminating fungi are derived from the egg shell

Bacterial contaminants are usually controlled effectively with penicillin and streptomycin added to the inoculum Fungi are much more difficult to eradicate, although mycostatin is often effective Slow-growing bacteria such as diphtheroids, certain types of micrococci and pleuropneumonialike organisms may be maintained inadvertently in serial passage with viruses under study. Their detection is sometimes difficult, and their presence may be the cause of reactions not attributable to the virus Nasopharyngeal washings and stool suspensions may carry herpes simplex virus Influenza and mumps may be encountered in throat washings in which other viruses are sought This happens very rarely and is of slight practical importance. The time-tested and reliable methods of filtration or isolation in particular tissues of susceptible hosts preliminary to propagation in chick embryos are in many instances of greatest value Adequate bacteriologic control and careful attention to details of technic are the best prevention against contamination.

TECHNICS OF INOCULATION

Chorio-Allantoic Membrane Inoculation

The chorio-allantoic membrane is best suited for the study of viral infections characterized by focal or pocklike lesions Of those infectious for man vaccinia, variola, herpes simplex and Newcastle disease viruses produce grossly visible pocks in the membrane. Several others, including influenza A and B, are inconsistent in this respect (Cox, 1952). The cytotropisms which typify these viruses are exhibited by their effect on each of the three germinal layers of which the chorio-allantoic membrane is composed

The outer ectoderm consists of chorionic epithelium derived from the early dorsal somatopleure The inner entoderm lines the rapidly expanding allantois which originates as a diverticulum of the embryonic hind gut The middle layer represents a fusion of chorionic and allantoic mesoderm It contains the membranal arterioles venules and their accompanying lymphatics An abundant capillary plexus develops in the chorionic mesoderm During the last week of incubation the membrane serves as a respiratory organ By the 14th or 15th day capillary loops from the chorionic plexus extend into the outer ectoderm in close proximity to the shell membrane (Romanoff, 1952) Exchange of gases takes place across these structures

Embryonated eggs of 10 to 12 days incubation are most satisfactory for chorio-allantoic membrane inoculation The inoculum is introduced on the membranal surface It may be exposed by the window method as originally described by Goodpasture and Buddingh (1935) A modification which creates an artificial air space was devised by Burnet (1936) Sealing the window by means of a cover glass resting on a ring of petrolatum-paraffin mixture or by replacing the shell sector is too time-consuming if transparent cellophane adhesive tape is available. A window is not essential if the area of the membrane to be inoculated is separated from the shell by gentle suction applied to a small drill hole over the air sac. A drill hole in the shell, including a slit in the shell membrane over the area to be inoculated, must be present in order to create the artificial air sac This opening also serves for the introduction of the inoculum which then may be distributed evenly by gently tilting the egg from side to side Good transillumination of the egg in a darkened room is required for this procedure Injections can also be forced blindly between the shell membrane

than carbohydrates provides the chief source of energy During this period tissue and organ differentiation and maturation progress rapidly Infectious agents introduced at this stage proliferate at ■ slower rate Inflammation becomes more intense, and the specific localizations of typical lesions which characterize the infection in the more mature natural host become apparent This is particularly marked from the 13th through the 15th days of incubation In what manner the more efficient utilization of energy and the accentuation of metabolic processes concerned with protein synthesis promotes these more specific and mature responses is not clear It is of particular interest that these phenomena become more apparent after the 12th or 13th day when the remaining egg albumen is absorbed into the amniotic cavity.

From the 15th day of incubation until hatching the cells of the yolk sac membrane become particularly active The mobilization and transport of fat from the yolk to the embryo constitutes one of the chief metabolic activities during this period It presumably anticipates the energy demands of hatching and the first few days of the life of the chick. The final stages of incubation are marked by an increasing insusceptibility to infectious agents not naturally pathogenic for the species

Throughout incubation the embryo appears to remain immunologically inactive Nonspecific factors such as complement begin to make their appearance about the 19th or 20th day. Specific antibodies in response to infection or following the introduction of antigens have not been detected by available methods Passive transfer of antibodies from actively immunized hens to the egg has been demonstrated repeatedly Thus, diphtheria antitoxin is concentrated in the yolk of eggs from hens actively immunized with diphtheria toxoid. Embryos developing in such eggs are protected against the action of the toxin. The transfer of bacterial and viral antibodies has also been shown to take place when laying hens recover from some infections or following certain vaccination procedures It is of interest to note that when poultry feeds containing antibiotics such as chlortetracycline, oxytetracycline and chloramphenicol are included in the diet of laying hens there results an increasing resistance of the eggs to infection with rickettsiae (Grieff and Pinkerton, 1951).

Propagation in the various substrates which characterize successive stages in the development of the embryonated egg has different effects on different viruses Adaptation may be achieved within a few passages or may require blind passage through several transfers. Diminution or loss of virulence for the natural host develops at varying rates Influenza virus, for instance, changes from the O to the D phase during a few passages and quickly loses its disease-producing capacity for man without much substantial change in its antigenic qualities. Vaccinia, on the other hand, remains relatively stable and becomes avirulent for human beings only after numerour transfers in the chorio-allantoic membrane. Strains of herpes simplex have been found to vary greatly in these respects In general, continued propagation exerts an attenuating influence on viruses for the natural host while it often increases their virulence for the chick embryo Other perhaps less immediately important variations are also induced. Most of these are considered to be the result of selective survival rather than due to true mutations Since viral replication involves the intracellular assembly of essential constituents it is not surprising that the resulting infectious agent soon or late develops new characteristics. These factors must be considered in relation to problems of antigenicity, serologic relationships, virulence and perhaps morphologic and chemical attributes of viruses adapted to the chick embryo by prolonged propagation.

THE CONTROL OF CONTAMINANTS

Endogenous Infections of the Egg. The most common bacteria transmitted congenitally through the egg are *S pullorum*, the cause of white diarrhea of chicks, and *S gallinarum*, of fowl typhoid Avian tuberculosis is also known to be transmissible in this way. These infections are unlikely to be a source of difficulty because of rigid inspection and control of flocks certified for breeding The potential danger of endogenous viruses has been reviewed by Cottral (1952). These include avian encephalitis, Newcastle disease,

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and the chorio-allantois through a small drill hole Uncertain results may be expected because of the ease with which the chorio-allantois is penetrated or torn. Melted paraffin or clear nail polish effectively seals the drill holes in the shell.

The ability to distinguish between specific and nonspecific lesions is acquired by experience gained from gross and microscopic comparisons of infected membranes with membranes receiving noninfectious control inocula. Slight injuries and hemorrhages caused by manipulative procedures give rise to most nonspecific lesions They usually are situated along the course of blood vessels and are irregular in outline and distribution Careful scrutiny reveals their mesodermal localization. Microscopically, they are composed of fibroblasts and histiocytes rather than inflammatory exudate. Particles of foreign or dead tissue in the inoculum may provoke nonspecific edema and inflammation Keratinization of the ectoderm evidenced by scaliness, wrinkling and focal thickening can be mistaken for specific reactions

Lesions due to infection are usually present in considerable numbers and are uniform in distribution and appearance In most instances they appear as definite blisters or pocks representing focal proliferations of ectodermal cells In later stages they become surrounded by inflammatory exudate and show central ulceration Areas of necrosis and hemorrhage are usually indicative of active infection

The microscopic features which characterize the early stages of infection provide the best indication of the specificity of membranal lesions. Reaction in the form of cellular hyperplasia, hypertrophy, vacuolar degeneration and necrosis confined predominately to one or another of the membranal layers is in many instances typical The recognition of intracytoplasmic or intranuclear inclusions within parasitized cells is helpful in determining the specific nature of lesions caused by several viruses Subinoculation with the production of typical reactions to infection in other species is often required for identification. Final characterization is established by neutralization of a virus by its specific antiserum or by means of other appropriate serologic reactions.

Allantoic Cavity Inoculation

The allantoic cavity is enclosed by the chorio-allantoic membrane It is lined by entodermal epithelial cells which support the replication of several important viruses Inoculation of the allantoic cavity is the method of choice for the study and the propagation of influenza virus types, A, B and D, mumps virus and Newcastle disease virus Cabasso et al. (1952) have adapted poliovirus type II to propagation in the embryo by this route The various products of the process of viral replication are shed into the allantoic fluid It has been estimated by various methods (Henle, 1953) that approximately 10^{7} entodermal cells line the allantoic cavity of the 11- to 12-day embryonated egg This has provided a valuable numerical basis for quantitative studies on the replication of influenza virus and the more exact delineation of its growth curve.

The allantoic sac serves as a repository for excreta from the embryonic kidneys (Romanoff, 1952). The fluid it contains is composed of a secretion from the entodermal lining to which the kidney excreta are constantly added. Its volume increases rapidly from about 1 ml on the 7th to 5 to 10 ml by the 13th incubation day. It then rapidly decreases and has almost completely disappeared at the time of hatching Allantoic fluid contains a high concentration of uric acid Between the 11th and the 13th days a rapid increase in the concentration of phosphates takes place At lower than incubator temperatures, the phosphates precipitate as urates giving the fluid a milky appearance

Inoculation of the allantoic cavity is easily accomplished by means of syringe and needle through a small drill hole in the shell. Removal of the fluid presents few difficulties and can be achieved by a number of different methods Influenza virus is readily propagated in this way in 10- to 12-day embryonated eggs. Allantoic fluid from infected eggs has been the source material for the preparation of specimens for morphologic studies, for chemical and physical determinations and for antigenic analyses of influenza virus Most of what is known regarding the mechanisms and the practical application of the hemagglutination phenomenon has been derived from studies with virus in or derived from allantoic fluid

The effect of hormones and of various therapeutic agents has been determined mostly by the allantoic route Influenza, mumps and Newcastle disease vaccines are prepared from allantoic fluid from embryonated eggs infected with the desired virus.

De-embryonated Eggs: The de-embryonated egg technic as introduced by Bernkopf (1949) represents a special adaptation of the use of the allantoic cavity By proper manipulation the embryo and the yolk sac are carefully poured out through a wide opening made in the air sac end of the egg The connections with the chorio-allantois are severed, leaving the membrane lining the inside of the egg shell. All remaining yolk, albumen and blood are thoroughly washed out with 3 changes of cold saline. The egg contents are replaced with 10 to 40 ml of Tyrode's or other suitable solution containing antibiotics in proper amounts. A sterile rubber cap seals the opening over the air sac The chorio-allantois will survive for several days under these conditions This method has proved to be extremely valuable for controlled studies of problems regarding details of the replication and growth curve of influenza virus (Henle, 1953)

Amniotic Cavity Inoculation

A variety of cells and tissues are accessible for infection by this route of inoculation The amniotic fluid brings infectious agents into contact with the epithelial cells of the inner lining of the amnion and the epidermal epithelium of the embryo In embryos older than 12 days localization of typical lesions and inflammatory reactions are likely to develop in different parts of the respiratory and the alimentary tracts because respiratory and swallowing movements carry amniotic fluid into these areas. Herpes simplex (Anderson, 1940), influenza virus (Burnet, 1940) and several bacterial infections such as *H pertussis, H influenzae, N meningitides* and *C diphtheriae* (Buddingh, 1952b) have been studied in this manner

The amniotic route of inoculation is the method of choice for the primary isolation of all types of influenza virus Once established at this site, most strains except those of type C are readily adapted to propagation by the allantoic route Burnet (1951) regards amniotic cavity inoculation as being of greatest importance for the study of influenza virus genetics and for its maintenance in the original or O phase

The amnion develops with the chorion from the ventral somatopleure. It envelops the embryo and joins over its posterior aspect The two folds of the membrane separate except at the line of fusion. The outer fold develops into the chorion, the inner becomes the amnion The line of fusion or sero-amniotic raphe develops a lumen about the 11th day This forms the pathway along which the remaining albumen enters the amniotic fluid about the 12th or 13th day Amniotic fluid appears soon after the membrane forms It gradually increases in amount to a maximum of 3 to [illegible] ml on the 13th and then diminishes rapidly after the 16th day

The fluid-filled amniotic sac functions as a protective shock absorber for the embryo The membrane is supplied with bands of smooth muscle which are in more or less constant contraction from the 4th through the 12th days, after which they gradually slow down This activity keeps the embryo in gentle motion and tends to prevent the formation of adhesions and other abnormalities Amniotic fluid attains its maximum specific gravity and greatest protein content following the entry of the albumen on the 13th or 14th day.

This route of inoculation has many potentialities which have not been adequately explored for the study of the pathogenesis of respiratory and alimentary tract infections Combined viral and bacterial infections established in the embryo by amniotic cavity inoculation have been studied recently (Buddingh, 1956) Amniotic fluid collected from 11- to 12-day embryos infected with herpes simplex virus by the yolk sac route on the 8th day provides an excellent source of virus for serologic and other studies This method may be applied with good effect to other viruses

Inoculation of the amniotic cavity is best accomplished through a window in the egg shell In this manner the course of the injecting needle can be controlled visually and the inoculum introduced into the cavity with certainty The pre-embryonic stab method performed through a small drill hole under strong transillumination is also effective but less

reliable. Blind stabs in the direction of the embryo are not to be depended on for proper inoculation by this route

Collection of the amniotic fluid is perhaps the most difficult of all of the chick-embryo technics Familiarity with the location and the characteristics of the surrounding amnion is required. The features which distinguish amniotic from allantoic fluid at different incubation stages must be recognized Before the 12th or 13th day amniotic fluid is water clear; after this the entry of the albumen gives it a slightly yellow color and viscid consistency. After the 11th day allantoic fluid may be distinguished by the presence of precipitated urates Amniotic fluid is best obtained by aspiration with a Pasteur pipette to which slight suction is applied Careful manipulation of the surrounding membranes with sterile forceps to prevent obstruction of the pipette is often necessary. This procedure requires considerable practice.

Yolk-Sac Inoculation

Inoculation into the yolk sac is in many ways the most satisfactory of all the chick-embryo technics The cells of the yolk sac are susceptible to all viruses which can be propagated in the embryo As first demonstrated by Cox (1938), it is especially useful for growing all types of rickettsiae The MEF1 strain of type II poliovirus has been adapted to chick-embryo propagation following yolk-sac inoculation by Roca-Garcia, Moyer and Cox (1952). This was achieved after the strain had been carried through numerous passages in suckling hamsters. Brueck and Buddingh (1951) found that the yolk sac was well suited to the culture of most pathogenic fungi. It has been used with good results for the propagation of many of the African and South American arthropod-borne viruses

The infected yolk sac is used for the preparation of rickettsial diagnostic antigens and for those of the psittacosis-lymphogranuloma group It serves as a practical means for the production of vaccines against epidemic and murine typhus, Rocky Mountain spotted fever, Q fever, equine encephalitis, Japanese B encephalitis and rabies. Infectivity titrations, the measurement of antibody content in serum and many therapeutic assays can be performed in the yolk sac (Cox, 1952).

The yolk sac develops from the extra-embryonic splanchnopleure as a continuation of the embryonic intestine and is the earliest extra-embryonic membrane. Its growth is extremely rapid, so that it encloses the entire yolk by the 6th day. A complex circulatory system develops from hemangioblasts at a very early stage. These form spaces which coalesce into vessels containing blood plasma and the true blood islands. Erythroblasts appear on the 2nd day, and white blood cells on the 3rd day. The yolk sac functions as a blood-forming organ and in the absorption of nutritive materials from the yolk and their transport to the embryo. Secretion of enzymes by the entodermal cells of the sac liquify the yolk and participate in its digestion

Variations in the type and the rate of metabolic activities of the yolk sac cells during different stages of incubation appear to influence susceptibility to viruses and the rate of their production This is evident from Crawley's (1948) studies on the comparison of LD50 mortality end points following yolk-sac inoculation with equine encephalitis virus on the 8th day and on the 15th day of incubation.

Removal of infected yolk sacs may be accomplished by any of several methods The sac may be grasped with sterile forceps through a wide opening over the air sac end of the shell The embryo may first be removed and the entire yolk poured out Excess yolk may be drained off, leaving the yolk sac membrane which may then be minced, ground, shaken with glass beads or broken up by other means whereby suspensions or preparations of the infectious agent are obtained Thin smears stained by the Giemsa or the Macchiavello method are helpful in the detection and the identification of rickettsiae. Special procedures are involved in the large-scale production of commercial vaccines.

Embryo Inoculation

Direct injection of the embryo by means of needle and syringe or through intravenous inoculation has been used for special purposes Intracerebral inoculation with direct visualization of the embryo through a window in the shell has been found to be applicable to the study of herpes simplex (Anderson, 1940) and rabies (Dawson, 1941). This route is

not essential for the propagation of these viruses, each of which is infectious by any route of inoculation. Injection into accessible areas of the embryo such as the eye, the nose, the peritoneal cavity or elsewhere is possible but for most purposes is unnecessary The embryonic stab method is useful for the introduction of yellow fever, Japanese B and equine encephalitis viruses This injection is performed under good transillumination, using a long needle by means of which the embryo can be penetrated

Several modifications of the original method of intravenous inoculation (Polk et al, 1938) have been developed Each was devised for special purposes. Intravenous injection is not widely used but has provided information regarding the dissemination of infectious agents introduced directly into the blood It has also been used to measure the effect of antiserum and chemotherapeutic agents

Under certain conditions dead embryos can be used to propagate influenza virus Lahelle and Horsfall (1949) demonstrated this with embryos killed by storage at room temperature for 7 to 10 days or at 4° C for 4 days Propagation of the virus was achieved at 35° C for 4 to 11 days after the embryos were killed Embryos frozen at −30° C for 20 hours were found to be unsuitable for this purpose Cox (1952) observed that a greater yield of spotted fever rickettsiae was obtained from a combination of living and dead embryos Seven-day embryos inoculated in the yolk sac usually die within 3 to 4 days The rickettsiae are found in abundance in the yolk sac but are few in number in the chorio-allantoic membrane. Further incubation for 2 to 4 days at 20° to 22° C will bring about a marked increase of rickettsial growth in the membrane. Only the rickettsiae of the spotted fever group behave in this manner, those of Q fever, typhus and scrub typhus do not

Foreign Tissue Grafts on the Chorio-Allantois

Human skin grafts obtained under aseptic conditions were first maintained on the chorioallantois by Goodpasture et al (1938). Human fetal membrane split into its component chorion and amnion were shown by Goodpasture and Anderson (1942) to take readily and to be susceptible to infection with several viruses The use of penicillin and streptomycin has greatly simplified these procedures by controlling most bacterial contaminants Human foreskin and other tissues can also be utilized Li et al (1955) have used this technic recently in attempts to adapt poliovirus strains to propagation in the tissues of the embryonated egg

Use of Eggs from Other Species

Because they are so readily available, domestic hen eggs are used almost exclusively for the propagation of viruses and rickettsiae The longer incubation period of 28 days gives duck and turkey eggs an advantage over the 21 days of the hen's egg for agents which proliferate slowly For this reason Peck et al (1955) have found duck eggs to be superior for the propagation of the virus in the production of rabies vaccine Species differences have been noted by Brandly (1937) in that the virus of infectious laryngotracheitis of fowls would propagate in embryonated hen and turkey eggs but not in those of ducks, guinea fowl or pigeons. Harris (1945) described infection of turtle eggs with vaccinia virus

REFERENCES

Anderson, K 1940, Pathogenesis of herpes simplex virus infection in chick embryos, Am J Path 16, 137-155

Bernkopf, H, 1949, Cultivation of influenza virus in the chorio-allantoic membrane of deembryonated eggs, Proc Soc Exper Biol & Med 72, 680-682

Beveridge, W I B, and Burnett, F M, 1946, The cultivation of viruses and rickettsiae in the chick embryo, Spec Rep Series Med Res Counc, London, No 256

Brandly, C A, 1937, Studies on certain filtrable viruses, factors concerned with egg propagation of fowl-pox and infectious laryngotracheitis, J Am Vet A 90, 479-487

Brueck, J W, and Buddingh, G J, 1951, Propagation of pathogenic fungi in the yolk sac of embryonated eggs, Proc Soc Exper Biol & Med 76, 258-261

Buddingh, G J, 1950, The culture and effects of viruses in chick embryo cells, in The Pathogenesis and Pathology of Viral Diseases, Kidd, John G (ed), New York, Columbia Univ Press

———, 1952a, The pathological effects of viruses on the chick embryo, Ann New York Acad Sc 55, 248-253

———, 1952b, Bacterial and mycotic infections of the chick embryo, Ann. New York Acad Sc 55, 282-287

Buddingh, G J, 1953, *Chick embryo technics, in* Viral and Rickettsial Infections of Man, Rivers, T M (ed), ed 2, Philadelphia, Lippincott

———, 1956, Experimental combined viral and bacterial infection (Influenza C and Hemophilus influenzae, type b) in embryonated eggs, J Exper Med *104, 947-958*

Burnet, F M, 1933, A virus disease of the canary of the fowl-pox group, J Path & Bact. *37, 107-122*

———, 1936, The use of the *developing egg in virus* research Spec Rep Series Med Res Counc, London, No *220*

———, 1940, Influenza virus infection of the chick embryo lung, Brit J Exper Path *21, 147-153*

———, 1951, Some biological implications of studies on influenza viruses II Reproduction and variation in influenza viruses Bull Johns Hopkins Hosp *88, 137-157*

———, 1953, Principles of Animal Virology, New York, Acad Press, *1955*

Cabasso, V J, Stebbins, M R, Dutcher, R M, Moyer, A W and Cox, H R, 1952 Poliomyelitis III Propagation of MEF1 strain of poliomyelitis virus in developing chick embryos by allantoic cavity inoculation Proc Soc Exper Biol & Med *81, 525-529*

Cottral, G E, 1952, Endogenous viruses in the egg, Ann New York Acad Sc *55, 221-235*

Cox, H R, 1938, Use of yolk sac of developing *chick embryo as medium for growing rickettsiae* of Rocky Mountain spotted fever and typhus groups, Pub Health Rep *53, 2241-2247*

———, 1952, Growth of viruses and rickettsiae in the developing chick embryo, Ann New York Acad Sc. *55, 236-247*

Crawley, J. F, 1948, Statistical analysis of factors affecting the susceptibility of chick embryos to eastern equine encephalomyelitis virus, J Immunol *59*, 83-94

Dawson, J R, 1941, A study of chick embryo adapted rabies virus, Am J Path *17, 177-188*

Gay, F P, and Thompson, R, 1929, Attempts to cultivate vaccine virus in growing chick embryo, Proc Soc Exper Biol & Med *26, 556-559*

Goodpasture, E W, 1933, Use of embryo chick in investigation of certain pathological problems, South M J *26*, 418-420

———, 1938, Some uses of the chick embryo for the study of infection and immunity, Am J Hyg *28, 111-129*

Goodpasture, E W, and Anderson, K, 1942, Virus infection of human fetal membranes grafted on the chorio-allantois of chick embryo, Am. J Path *18, 563-575*

Goodpasture, E W., and Buddingh, G J, 1933, Human immunization with a dermal vaccine cultivated on the membranes of chick embryos, Science *78*, 484-485.

———, 1935, The preparation of anti-smallpox vaccine by culture of the virus in the chorio-allantoic membrane of chick embryos and its use in human immunization, Am J. Hyg *21, 319-360*

Goodpasture, E W, Douglas, B, and Anderson, K, 1938, Study of human skin grafted upon the chorio-allantois of chick embryos, J. Exper Med *68*, 891-*904*

Goodpasture, E W, Woodruff, A M, and Buddingh, E J, 1931, The cultivation of vaccine and other viruses in the chorio-allantoic membrane of chick embryos, Science *74, 371-372*

Greiff, D, and Pinkerton, H, 1951, Rickettsiostasis in fertile eggs from use of antibiotic residues in *poultry feeds, Proc Soc Exper Biol. & Med 78,* 690-692.

Harris, P N, 1945, Vaccinal infection of the chorio-allantoic membrane of the turtle embryo, Am J Path *21, 377-385*

Henle, W, 1953, Multiplication of influenza virus in the entodermal cells of the allantois of the chick embryo, *in* Advances in Virus Research, vol 1, New York, Acad Press

Jouan, C, and Staub, A, 1920, Etude sur la peste aviare, Ann Inst Pasteur *34, 343-357*

Lahelle, D, and Horsfall, F L, 1949, Multiplication of influenza virus in dead chick embryos, Proc Soc Exper Biol & Med *70, 547-551*

Levaditi, C, 1906, La spirillose des embryons de poulet, Ann Inst Pasteur *20, 924-938*

Li, C P, Schaeffer, M, and Nelson, D B, 1955, Experimentally produced variants of poliomyelitis virus combining in vivo techniques, Ann New York Acad Sc *61, 902-910*

Peck, F B, Jr, Powell, H M, and Culbertson, C G, *1955,* A new antirabies vaccine for human use, clinical and laboratory results using rabies vaccine made from embryonated duck eggs, J Lab & Clin Med *45, 679-683*

Polk, A D, Buddingh, G J, and Goodpasture, E W, 1938, An experimental study of complement and hemolytic amboceptor introduced into chick embryos, Am J Path *14, 71-86*

Roca-Garcia, M, Moyer, A W, and Cox, H F, 1952, Poliomyelitis II Propagation of MEF1 Strain of Poliomyelitis virus in developing chick embryos by yolk sac inoculation Proc Soc Exper Biol. & Med *81, 519-525*

Romanoff, A L, 1952, Membrane growth and function, Ann New York Acad Sc *55, 288-301*

Rous, P, and Murphy, J B, 1911, Tumor implantation in the developing embryo Experiments with a sarcoma of the fowl, J A M A *56, 741-742*

Smith, W, 1935, Cultivation of the virus of influenza, *Brit J Exper Path 16, 508-512*

Stevenson, W D H, and Butler, M B E, 1933, Dermal strains of vaccinia virus grown on the chorio-allantoic membrane of chick embryos, Lancet *225, 228-230*

Woodruff, A. M., and Goodpasture, E. W, 1931, The susceptibility of the chorio-allantoic membrane of chick embryos to infection with the fowl-pox virus, Am J Path *7, 209-222*

Zia, S, 1934, The cultivation of Mexican and European typhus rickettsiae in the chorio-allantoic membrane of the chick embryo, Am J Path *10, 211-218*

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9

Tissue-Culture Technics Employed in the Propagation of Viruses and Rickettsiae

It is not surprising that attempts to propagate animal viruses and rickettsiae in isolated systems of cells followed the development by Harrison, 1907, of a simple method of tissue culture For it was early apparent to most observers that these agents, unlike bacteria, fail to multiply in lifeless media The possibilities of this new technic for their cultivation were foreshadowed in the experiments of Steinhardt et al, 1913, who showed that the virus of vaccinia at least survived for several weeks in cultures of the corneal tissue of guinea pigs and rabbits However, they did not obtain unequivocal evidence that multiplication occurred, although their results suggested that infectivity had increased 6 to 10 times. This magnitude of increase is not sufficient to indicate multiplication Indeed, 12 years elapsed before Parker and Nye, 1925, presented data that removed any doubt regarding the capacity of viruses to multiply in cultures of tissue cells These investigators carried vaccinia virus through a series of 11 cultures of rabbit testicular tissue and found, in the last, 51,000 times as much virus as in the initial preparation Since this demonstration many viruses and rickettsiae pathogenic for man and lower animals have been maintained by serial passage in tissue culture Lists of most of the mammalian viruses reported to have been cultivated up to about 1950 will be found in the reviews of Robbins and Enders (1950), Lynn and Morgan (1954) and Sanders et al (1953). Since the beginning of the present decade, as recorded in other sections of this book, a large number of others, including agents such as those comprising the Echo and the Adenovirus groups which represent hitherto unrecognized species, have been propagated in tissue cultures Many of these have been listed by Enders (1954) and Ross and Syverton (1957)

Although in the past the method was applied effectively to the investigation of several fundamental problems relating to animal viruses, during a long period it did not appear to offer the advantages for their cultivation and study provided by the susceptible animal, since it was considered that viral increase in cell systems could be demonstrated reliably only by inoculation of appropriate animals with constituents of the culture Recently, however, the tissue culture has assumed the status of a major technic in the virus laboratory Three circumstances, at least, accounted for its present importance

The first was the general recognition by virologists about 1950 that many viruses as

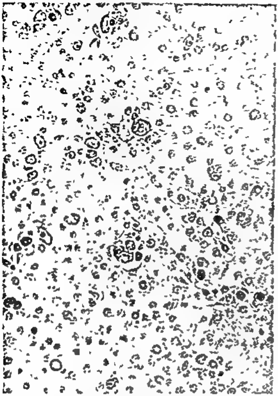

FIG 45 Primary monolayer culture on glass 37 days after trypsinized human amnion cells were placed in the tube. Trypan blue (final concentration 0 02%) was added to the culture for 10 minutes shortly before the photograph was taken. (×160)

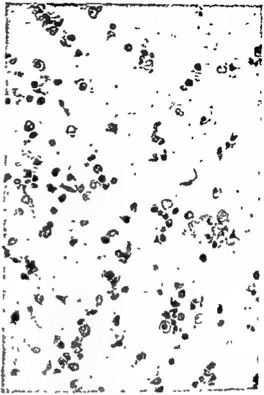

FIG 46. Appearance of human amnion cells in a culture comparable with that shown in Figure 45, 8 days after addition of 70 ID_{50} of poliovirus Type I (Mahoney strain). Note rounding, shrinking, nuclear pyknosis and disappearance of many cells (×160)

they multiply produce degenerative changes in cultured cells which can be easily distinguished This phenomenon had been described previously by a few workers (Topacio and Hyde, 1932, Huang, 1942), but its significance was not widely appreciated until about this time when it became obvious to all that with these so-called "cytopathogenic" viruses resort to the living animal was unnecessary. For, as was emphasized in the case of poliomyelitis (Robbins et al, 1950), the changes that they produce within cultured cells provide adequate criteria of their presence. Examples of these changes are illustrated by Figures 45, 46 and 47. Furthermore, it was determined contemporaneously that the addition of specific antibody to the culture along with the viral inoculum prevented the development of the cytopathic effects Accordingly, it became evident that the experimental animal could also be replaced by the culture in the demonstration and the assay of antibodies and conversely in the serologic identification of the agents themselves.

The second circumstance responsible for the present importance of the tissue culture was the development of antibiotics The inclusion of these substances in the medium made it possible to prepare and apply tissue cultures on a virtually unlimited scale. Before antibiotics were available the number and the size of cultures that could be established and maintained was greatly limited because of the necessity of exercising at every step the most stringent precautions to avoid bacterial contamination Moreover, the incorporation of antibiotics in the medium permitted the direct isolation of viruses in tissue cultures

from unfiltered fecal suspensions, throat washings and other materials heavily contaminated with bacteria

Finally, the usefulness of the method was greatly enhanced by the revival in 1952-53 of a technic devised many years earlier by Rous and Jones (1916) for the preparation of suspensions of tissue cells by treatment with trypsin From such suspensions large numbers of uniform cultures consisting of a single sheet or monolayer of cells can be prepared with great ease and uniformity

Accordingly, for many purposes both practical and investigative, tissue culture technics have supplanted those involving the use of living animals, including chick embryos, because they are more rapid, equally or more accurate and sensitive, and are applicable to a wide range of problems, some of which cannot be investigated as satisfactorily in the more complex milieu of the living animal

In this chapter the principal procedures employed in the past for the cultivation of viruses and rickettsiae in cell systems are described briefly, while those introduced during the last few years and now adopted as routine for many purposes are presented in somewhat more detail It is emphasized that the principal aim of these technical descriptions is simply to aid the reader to visualize the actual procedures so that he will be able to appreciate more readily the significance of results described in other chapters that have been obtained through the application of the tissue culture method In no sense, therefore, does this chapter represent a practical manual of tissue culture Those intending to use any of the technics outlined should consult one of the most recent treatises such as that of Melnick (1956), compiled especially for the laboratory worker, or the original articles to which references are given

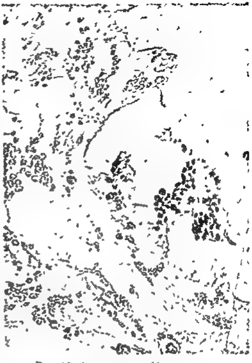

FIG. 47. Appearance of human amnion cells in a culture comparable with that shown in Figure 45, 17 days after addition of 200 ID_{50} of measles virus (Edmonston strain) Note fusion of cells into a large syncytial-like mass which includes numerous aggregates of nuclei and contrast this cytopathic effect with that produced by poliovirus (×160)

TECHNICS OF TISSUE CULTURE EMPLOYED FOR THE PROPAGATION OF VIRUSES AND RICKETTSIAE

INTRODUCTION

Various procedures devised primarily for the cultivation of tissue cells have been adapted afterward to the propagation of viruses and rickettsiae In a few instances technics have also been invented with these special purposes in mind Although they differ widely in details, the essential elements of all consist of living cells and a physiologic medium for their support The original source of the cells is, of course, the tissues of the animal body Tissues for primary culture may be derived from the normal individual at any stage of development—embryonic, fetal, youthful or aged—or from neoplasms The composition of the medium may vary from a simple solution of salts and glucose to one consisting of a complicated mixture of known nutrients and accessory growth factors or one composed of tissue juices, blood serum or other body constituents in which the components are chemically undefined Depending

upon the composition of the medium, cells may survive for a time without multiplying, or they may undergo active multiplication which may continue indefinitely or cease after a while Solutions that merely permit survival are referred to as "maintenance" media; those providing factors necessary for multiplication are designated "*growth-promoting*" media

Classification of Technics

"Tissue" and "Cell" Cultures. For convenience in presentation, the various kinds of cultures employed in work with viruses and rickettsiae can be separated into two main groups (Table 8) Group I includes cell systems established with tissue fragments or with masses of cell outgrowth originating therefrom Cultures of this sort have been in use for many years Group II includes cell systems initiated with a suspension of dispersed cells As already stated, the technic for the dispersion of tissue cells was described in 1916 by Rous and Jones Long neglected, during the last few years it has become for most purposes the procedure of choice in the virus laboratory. Cultures prepared with dispersed cells have recently been termed "cell cultures" by Ross and Syverton (1957) to distinguish them from those included in Group I

The various modifications based upon these two basic procedures can be divided in each case into 2 subgroups as shown in Table 8. One comprises systems in which tissue fragments or cells are supported in or on a semisolid or solid substrate The substrate may consist of a variety of materials that include the vessel wall itself, fibrin generated from blood plasma, agar, cellophane, or cellulose strands, etc To the other belong those systems in which fragments or single cells are not fixed to a substrate but remain suspended in the nutritive medium

Procedures and Materials Commonly Employed in Tissue and Cell Cultures

As a necessary background to descriptions that follow of the different kinds of cultures mentioned in Table 8, an outline of the manner in which the tissues, the cells and other constituents in general are selected, handled or prepared is presented here.

Prevention of Contamination by Micro-organisms. Strict precautions to exclude contamination by bacteria and fungi are observed at every step. In the modern virus laboratory, as previously indicated, elimination of micro-organisms derived from the air or other sources usually is accomplished effectively by the addition of antibiotics such as penicillin, streptomycin and mycostatin to the medium. Formerly, in the prevention of airborne contamination, reliance was placed chiefly on the performance of all manipulations under conditions which reduced the bacterial content of the air to a minimum (Parker, 1950) While these measures are now not essential, their use, if facilities are available, adds a desirable factor of safety Although in most instances the antibiotics commonly employed prevent the development of micro-organisms that may be initially present in the constituents of the culture, their effectiveness cannot be taken for granted, because resistant bacteria or fungi are encountered occasionally Therefore, it is necessary to test all constituents by inoculation into suitable growth media before they are combined in the culture system

Tissues and Cells

1 Sources Tissues for primary explants are obtained from freshly killed animals, from organs removed at operation, from embryonated eggs, from embryos or fetuses taken after death of the mother or at operation, or when delivered prematurely and from fetal membranes at the time of normal delivery. At present in many laboratories stock cultures of established cell lines are maintained from which subcultures to be used for viral propagation are prepared * A few of these ... from a single

available lately, only a few have as yet been investigated extensively in respect to their reactions with a large number of viruses. Of these lines one of the oldest was developed by Earle (1943) from murine tissue and is designated the "L" strain of mouse fibroblasts Its capacity to support multiplication of various species of human viruses is limited (Scherer, 1953) A broader spectrum of susceptibility to viruses pathogenic for man is exhibited by the so-called HeLa strain (Scherer and Syverton, 1954a, 1954b, 1955), initiated by Gey and his associates (1952) from human cervical carcinoma tissue. There-

* An "established" line or strain consists of homogeneous-appearing populations of cells that have been propagated by serial explantation over a prolonged period

TABLE 8 "TISSUE" AND "CELL" CULTURE TECHNICS USED IN THE PROPAGATION OF VIRUSES AND RICKETTSIAE

Group I						Group II					
Fragments of Tissues or Cell Outgrowth						Dispersed Cells					
Suspended Fragments	Fixed Fragments or Cell Outgrowth					Suspended Cells	Fixed Cells				
	Plasma Clot		Roller Tube	Glass	Agar		Monolayer on Plasma or Glass	Monolayer on Glass and Agar Overlay	Agar	Clonal Growth	
	Drop	Flask								On Glass	On Feeder Monolayer
Maitland and Maitland (1928) Li and Rivers (1930) Plotz (1917)	Harrison (1907)	Carrel (1923)	Gey (1933)	Gey and Gey (1936) Melnick (1933)	Zinsser et al (1939)	Owens et al (1954) Earle et al (1954) Cherry and Hull (1956) McLimans et al (1957a)	Rous and Jones (1916) Lesch and Jentoft (1953) Scherer et al (1953) Youngner (1954)	Dulbecco and Vogt (1954)	Wallace and Hanks (1958)	Puck, Marcus and Cieciura (1953)	Puck and Marcus (1955)

fore, it has been adopted by many laboratories as routine for diagnostic and investigative purposes. Other available strains are the epithelial-like cells developed by Chang (1954) from different fetal tissues, those originated by Berman and his co-workers (1955) from human bone marrow and the so-called "KB," "H Ep" and "Maban" lines of human carci-

amnion and other normal human tissues, and Salk and Ward (1957) have described a line derived from monkey heart tissue

2. SELECTION OF SUSCEPTIBLE CELLS As indicated, cells derived from many animal species and from different organs have been employed in the cultivation of viruses. Embryonic cells tend to grow more rapidly and vigorously than those of more mature individuals For this reason and because they may be obtained easily, chick-embryo tissues were frequently selected by earlier workers. Because they are readily available, primary explants of the tissues of small laboratory animals such as rabbits, guinea pigs and mice were also used extensively in the past

While the cells of lower animals are still often employed in the cultivation of certain viruses, it gradually became apparent that a number of agents associated with human diseases, for example poliovirus and the agent of varicella, fail to increase in them In 1949 it was shown (Enders et al., 1949, Weller et al, 1949) that polioviruses multiply readily in cultures of different human tissues Subsequently, investigations have demonstrated the capacity of human and other primate cells to support the growth of viruses that behave in an analogous manner (cf Ross and Syver-

man Similarly, it has been emphasized lately that agents pathogenic for lower animals, e g, the virus of canine hepatitis (Fieldsteel and Emery, 1954), which do not multiply in cells of unrelated animals can be cultivated easily in those of the same species The success of contemporary researches on certain viruses of importance in veterinary medicine thus depended upon increasing recognition of this fact.

Although it is clear that marked differences are encountered in resistance of cells from different species when they are first explanted, there are indications that these differences may be obliterated after prolonged cultivation in vitro. For example, Gey and Bang (1951) found that an established line of normal rat fibroblasts was completely resistant to eastern equine encephalomyelitis virus In contrast, a strain of malignant cells derived from this normal line not only supported multiplication of the agent but was completely destroyed by it. Sheffield and Churcher (1957) have shown lately that a line of rabbit embryonic kidney cells established by Westwood and his co-workers (1957) eventually become susceptible to infection by poliovirus Therefore, it would seem possible that the susceptibility to viruses of cells of other established lines may be found occasionally to depart widely from that characteristic of the progenitors of the line.

Resistance or susceptibility of cells from the same species may also be correlated with morphologic type as demonstrated by several investigators An illustration of this fact is provided by Stulberg and Schapira (1953) who found that influenza A virus multiplied actively in epithelial cells from the chick embryo's lung. In fibroblasts from the same source no evidence of increase was obtained Furthermore, variants of the same viral species may exhibit differences in their capacity to multiply in or injure the same kind of cell (Jervis, 1955, Enders, 1957).

Because of these variations in cellular resistance it is obviously essential to select susceptible cells for the cultivation of a given virus When the nature of the virus is unknown, it is equally evident that cultures of cells of different types from the natural host or one closely related should be employed initially.

3. HANDLING OF TISSUES In the removal of tissues from the animal body or the egg, care is taken to avoid crushing and to prevent the tissue from coming in contact with chemical disinfectants or other materials injurious to cells As soon as possible after removal, the tissue is cut into fragments which are placed in a maintenance medium.

4. STORAGE. Tissue fragments or suspensions of dispersed cells, as well as completed cultures, can be stored for considerable periods under suitable conditions The latter may vary according to the nature of the material Many kinds of cells survive and are capable of subsequent multiplication after storage at 4° to

even

or 32

prolonged periods, provided that a nutrient medium is supplied While metabolic processes continue under these circumstances, cell division is greatly reduced At temperatures of $-60°$ to $-70°$ C. cells suspended in a medium containing glycerine and quickly frozen may be preserved for months (Scherer and Hoogasian, 1954).

5 Media As noted, numerous media of widely varying chemical composition have been employed to maintain the viability or promote the active multiplication of cells in culture Taken as a whole, media that merely preserve for a time cell viability and metabolism are less complex than those which provide factors essential for active and continuous cell propagation Thus, certain cells may survive for periods of several weeks in dilute mixtures of a few inorganic salts and glucose often referred to as "balanced salt solutions," although under these conditions little or no multiplication takes place The prototype of such mixtures is Tyrode's solution which has the composition given in Table 9. In tissue cultures used for the propagation of viruses it was first employed by Li and Rivers (1930) and subsequently has been modified in various ways with the purpose of increasing its efficiency as a cell-sustaining medium Balanced salt solutions of the composition devised by Earle (Table 9) and Hanks (Melnick, 1956) are among those now in more common use Included as routine in these simple solutions as well as in more complex media is an indicator dye, usually phenol red, which serves to detect changes in pH that are regularly brought about by metabolizing cells in closed systems When the latter are killed by viruses or other agents the pH remains constant

Occupying a position intermediate between the salt mixtures and the complete growth-promoting media are those of definite but complex chemical composition In the case of nearly all cells that have been examined, mixtures of this kind may support growth as well as preserve cell viability over varying periods Eventually, however, metabolism and growth diminish An exception is presented by a clonal line of strain L cells which Evans and her co-workers (1956) showed to be capable of continued active multiplication in a medium of defined but highly [illegible]

[illegible] addition to the components of

Table 9 Composition of Tyrode's and Earle's Salt-Glucose Solutions (aqueous) *

Constituents	Grams per Liter	
	Tyrode	Earle
NaCl	8 00	6 80
KCl	0 20	0 40
$CaCl_2$	0 20	0 20
$MgCl_2 \cdot 6H_2O$	0 10	—
$MgSO_4 \cdot 7H_2O$	—	0 20
$NaH_2PO_4 \cdot H_2O$	0 05	0 14
$NaHCO_3$	1 00	2 20
Glucose	1 00	1 00

* From Parker (1950)

Earle's solution it contains many of the biochemical factors known to be involved in cellular metabolism These include 19 amino acids, glutamine, vitamins A, D, E, K and the B group, biotin, folic acid, cholesterol, purines, pyrimidines, ribose, desoxyribose, adenylic acid, adenosinetriphosphate and ferric nitrate.

While defined media of this sort may be required, or at least may give better results with certain kinds of cells, it is apparent that with others the number of components can be much reduced For example, Rappaport (1956) consistently observed limited multiplication and prolonged survival of monkey renal cells in a mixture of inorganic salts, 5 trace elements, glucose, ribose and 7 amino acids

Under certain circumstances these media are preferable to either salt-glucose solutions or the complete growth-promoting media They may provide factors lacking in the simplest media that are essential in viral multiplication or at least greatly enhance it As compared with complete media, the absence of serum and tissue proteins is advantageous for many reasons Thus, such substances may interfere with infection of the cells by virus from infected culture fluids, impede accurate investigation of biochemical factors involved in viral synthesis and preclude their use in the manufacture of vaccines, because they may induce protein hypersensitivity or serum hepatitis in the recipient

Mixtures capable of supporting cellular multiplication indefinitely were devised soon after Harrison's development of the tissue culture. Details of the preparation of the various constituents as well as information

regarding the proportions in which these may be combined will be found in Parker's monograph (1950) Qualitatively and quantitatively, the composition of complete growth media has varied greatly, as will be evident from even a cursory glance through the references to media listed in Murray and Kopech's monumental bibliography of the literature on tissue culture (1953). All contain as an indispensible constituent animal protein or chemically ill-defined break-down products of animal protein. Accordingly, although it has been pursued long and actively, the formulation of a medium of defined chemical composition that will support continuous cell multiplication, with the exception noted above, has not yet been achieved However, the recent formulation of solutions such as those of Waymouth (1956), Healy et al. (1955), Evans and her associates (1956) and Eagle (1955b) appear to have brought us much closer to this important objective

Most of the complete media employed in the cultivation of viruses or rickettsiae have consisted essentially of blood plasma or serum or a combination of these and an extract of embryonic or adult tissue Balanced salt solution is usually added in varying proportion to these basic constituents

Fowl plasma, although representing a heterologous element in cultures of mammalian cells, usually has been selected because the fibrin clot derived from it is firmer, more transparent and more resistant to lytic enzymes produced during cell growth than those from mammalian plasma Although plasma may furnish the only source of nutritive materials, as in the plasma hanging-drop culture, in other systems it serves to attach tissue fragments to the vessel wall and provides a favorable surface for migration and growth of cells

Blood serum of mammals and birds, as long recognized, contains unknown substances requisite for the continuous growth and multiplication of most cells. Lately this fact has been strongly re-emphasized by Eagle's (1955) experiments on the amino acid and vitamin growth requirements of L strain and HeLa cells. Although Eagle showed that each of 13 amino acids and 7 vitamins were essential as well as certain other substances of definite composition, undefined factors supplied by the dialysed serum also proved to be indispensible. Whereas these factors may be found in other proteins, usually they have been supplied most conveniently in the form of blood serum. Growth of many cells will take place in media containing sera of heterologous species However, in certain cases, [illegible]

cells may be adapted to a medium containing heterologous sera. Even more rigid requirements in respect to the nature and the origin of the serum are exhibited by some cells as exemplified by Chang's (1954) experiments on the cultivation of lines of human embryonic conjunctival, renal and hepatic cells For a prolonged period following their establishment in vitro these cells proliferated actively only in sera derived from selected human donors.

Extracts of various organs of postnatal animals as well as of embryonic tissues have long been known to contain factors that stimulate the multiplication of cells in vitro. Chick-embryo extract, because of its activity and [illegible]

primary or early explants, extracts of beef embryo tissues are superior The chemical properties of the growth-promoting substances in tissue extracts are largely unknown, in spite of many attempts made to define them

Descriptions of Various Tissue Culture Technics

With this sketch in mind of the components used in various tissue cultures the technics whereby they are assembled into systems of surviving or proliferating cells are described.

Technics of Group I—Fixed Fragment Cultures. The technics included in Group I (Table 8) are considered first, beginning with those of the main subgroup in which the tissue fragments or mass of cell outgrowth are fixed to the vessel wall In 3 of these systems fixation is accomplished by means of a fibrin matrix generated from plasma Of these, 2 are now chiefly of historical interest to the virologist. Thus while the plasma or hanging-drop culture represents the prototype of the tissue culture as devised by Harrison, 1907, it offers no advantages over the more versatile roller-tube fragment culture The flask culture as developed by Carrel (1923) is to be recalled because it permitted the propagation of larger quantities of cells under conditions that could be controlled more precisely. Investigations carried out by means of the Carrel-flask culture led

to many technical improvements of importance such as the use of tissue extract to promote rapid and continuous cell multiplication. The technic of the roller tube fragment culture was perfected largely by Gey (1933) and by Lewis (1935), although the underlying principle was recognized earlier. While retaining most of the advantages of the Carrel flask, in addition it permits cultivation of large amounts of tissue which may be easily maintained and handled with the need for little equipment other than that found in any microbiologic laboratory. However, except for special purposes, it has been superseded lately by the dispersed cell culture. For complete descriptions of the plasma-drop and the Carrel-flask technics Parker's monograph (1950) should be consulted. Details of the roller-tube technic are given by Melnick (1956).

1. Plasma-Drop Cultures. In the preparation of primary explants the tissue is cut into fragments about 1 mm in the longest dimension. Then a fragment is centered in a drop of medium which previously has been placed on a coverslip and usually consists of a mixture of equal parts of fowl plasma and balanced salt solution. After the plasma coagulates, the preparation is inverted over the cavity of a depression slide. The coverslip is fixed at the edges to the slide with paraffin. The preparation may be examined under the oil-immersion lens in the fresh state, or sections may be cut after fixation and staining. Within a few days at 37° C a mass of cell outgrowth occurs about the periphery of the fragment. From this outgrowth secondary explants may be initiated by dividing it into sections and transferring each section to a plasma drop. Within a short time the cells in these "explants" increase to such an extent that further subdivision and explantation is required.

2. Carrel-Flask Cultures. Because of certain disadvantages inherent in the plasma-drop method (small amount of cells, necessity for frequent explantation, difficulty in sampling or altering the menstruum surrounding the cells), Carrel devised a system in which cells could be maintained for much longer periods without explantation. This system consists of cells, plasma coagulum, nutrient medium and an overlying gas-mixture. The culture is established in a flat-walled circular flask in which a shallow layer of medium bathes the cells which are attached by a thin layer of fibrin to one side of the vessel. Under these conditions, renewal of the medium, changes in its constituents, adjustment of pH and replacement in the gaseous phase are accomplished easily. Many studies were made of the effects on cells of changes in the fluid and gaseous phase and conversely of the effect of the latter on the growth of the cells. However, this kind of culture was never applied extensively to the propagation of viruses and rickettsiae, probably because much skill and care was required in its preparation and maintenance previous to the antibiotic era.

3. Roller-Tube Cultures. The essential constituents are the same as those included in Carrel-flask cultures. Although ordinary test tubes (150 × 15 mm) are used as routine, other vessels of various shape and size have been adopted for special purposes, such as the production of large quantities of virus. In the preparation of a roller-tube culture a drop or two of plasma is spread over the lower half of the inner surface of the tube. The tissue fragments, of which there may be 20 or more, are distributed uniformly throughout the plasma film. After the latter coagulates, about 2 ml of medium is introduced and the tube is closed with a rubber stopper. Then it is placed at a slight angle from the horizontal in a slowly rotating drum enclosed in an incubator. When the medium becomes acid it is changed. Explants can be made if required. Unless special thin-walled tubes are used, microscopic examination of the cellular outgrowth is possible only under the low-power lens. However, after fixation, embedding in collodion and staining, the entire contents of the culture can be studied in detail (Enders and Peebles 1954). This type of culture is among those well suited to investigations of the effects of prolonged association between cells and viruses, since by regulating the conditions, it is possible to preserve cell viability indefinitely.

4. Fragment Cultures on Glass and Agar. The plasma clot possesses certain disadvantages as a cell support. It tends to become cloudy as the culture ages, rendering difficult the visualization of cellular details, or it may liquefy with consequent detachment of the cells. Moreover, studies of biologic and

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Extracts of various organs of postnatal animals as well as of embryonic tissues have long been known to contain factors that stimulate the multiplication of cells in vitro. Chick-embryo extract, because of its activity and because it can be prepared easily, early came to be regarded as the standard. However, for the cultivation of some mammalian cells in primary or early explants, extracts of beef embryo tissues are superior. The chemical properties of the growth-promoting substances in tissue extracts are largely unknown, in spite of many attempts made to define them.

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formalinized vaccine used in the Poliomyelitis Vaccine Field Trial of 1954. Essentially the same procedures are still employed by certain manufacturers of the vaccine. To large rectangular bottles (5 liter capacity) Farrell and his associates added approximately 5 Gm. of minced kidney tissue from rhesus or cynomolgus monkeys and 500 ml. of Mixture 199. The bottles were rocked mechanically at 37° C during a period of 1 to 6 days, then the medium was removed and replaced with fresh material. Poliovirus was introduced, and agitation and incubation were continued. Within 2 to 7 days thereafter a large increase in the agent occurred as indicated by titration of the fluid phase. The low protein content of the fluid (0.25-0.30 mg. N/ml.) rendered the material especially suitable as a source of virus for the preparation of vaccine.

Technics of Group II—Fixed-Cell Cultures. The procedure devised by Rous and Jones (1916) for the preparation of dispersed suspensions of tissue cells by treatment with dilute trypsin was reintroduced by Scherer et al. (1953), Frisch and Jentoft (1953), Dulbecco and Vogt (1954) and Youngner (1954). The first group of workers employed it to remove from the glass and disperse aggregates of HeLa cells maintained in continuous cultures; the others applied it to the preparation of cell suspensions from tissue fragments. The suspensions are largely composed of single cells, although usually a varying number of small aggregates are included. When allowed to settle on a glass surface the cells rapidly adhere and in a suitable medium at 37° C increase in size and multiply. Eventually through their approximation, a monolayer is formed in which cytopathic changes induced by viruses or other agents can be visualized with great ease and clarity. It is possible with dispersed-cell suspensions to prepare rapidly and conveniently large numbers of uniform cultures. Formerly, methods for the enumeration of the cells introduced as well as the final cell population were unsatisfactory on the whole. The availability of dispersed-cell suspensions allows the experimental control of quantitative relations between cells and virus to a degree heretofore unattainable. Indeed, with cells from a trypsinized suspension it has even been possible to determine the rate and the quantity of virus produced by a single infected cell while observed under the microscope (Lwoff et al., 1955).

The development of the technic of preparing cell suspensions, together with the recognition of the cytopathic changes produced in vitro by viruses, has put in the hands of animal virologists the essential means for the quantitative study of cell-virus relationships which students of bacteriophages have long employed and has made possible many fundamental advances in knowledge.

Trypsinization of a variety of human and animal tissues—mature, embryonic and malignant—yields suspensions of fibroblastic and epithelial-like cells that readily form monolayers. Among these tissues, kidney and amniotic membrane have so far been employed most extensively. The procedure as described by Dulbecco and Vogt (1954) and Youngner (1954) has been modified subsequently in details by other workers with a view to increasing the cell yield and decreasing the number of manipulations. The modification proposed by Bodian (1956) fulfills these aims satisfactorily and will be given here as an example of the method.

The minced kidney cortical tissue from one monkey is placed in an Erlenmeyer flask with indentations at the base which serve to increase the efficiency of the process. Then 150 ml. of 0.25 per cent trypsin in Hanks' solution at pH 7.5 is added. With a magnetic stirrer the contents are mixed at a rate just short of bubbling at 4° C for 6 hours or at 22° to 26° C for 2 hours. The supernatant fluid that contains products toxic for cells is then removed and replaced with fresh trypsin solution. Stirring at 4° C is continued for 16 to 20 hours. The supernatant fluid containing the dispersed cells is removed from tissue residues and is slowly centrifuged (600 rpm) for 5 minutes. The packed cells are washed repeatedly in Hanks' solution and finally resuspended in sufficient growth medium to yield a suspension of about 300,000 cells per ml. This adjustment is made by counting the cells in a hemocytometer at the time of the last washing.

1. Simple Monolayer Cultures on Glass. At the present time the preparation of monolayer cultures consists simply in adding definite volumes of the cell suspension to

biochemical reactions of the cells are made difficult or impossible by the presence of the coagulum Finally, it may prevent uniform dissemination of virus throughout the cell population Therefore, the value of other substrates has been explored intensively Among those deserving particular mention are glass and agar Early work by Gey and Gey (1936) showed that cells could be propagated directly on glass In 1953, Morann and Melnick described a method for cultivating monkey kidney epithelial cells in this manner. By preheating the culture tubes at 45° C they found that the tissue fragments in the absence of excess fluid become adherent to the glass After a short interval at 40° C. the medium can be added without displacing the fragments. Then the cultures are left undisturbed for 10 to 14 days at 36° C, by which time sheets of epithelial cell outgrowth become established about many of the fragments This, like other fixed-fragment cultures, has been almost completely replaced by the dispersed cell culture

In 1937, Zinsser and his co-workers seeking a simplified technic for cultivating large quantities of typhus rickettsiae to be used in the manufacture of vaccine, distributed numerous fragments of chick-embryonic tissue on the surface of isotonic agar slants containing horse serum and Tyrode's solution Thus supported and nourished, the cells remained viable for several weeks Several species of rickettsiae as well as a number of viruses multiply actively under these conditions

Technics of Group I—Suspended Fragment Cultures. A system was described by the Maitlands in 1928 that offered to virologists greater simplicity and ease in preparation than most of those which have been described Therefore, it was adopted immediately and with various modifications was widely applied during the ensuing 20 years to the cultivation of viruses and rickettsiae. As with fixed-fragment cultures modern advances in technics have led to its virtual abandonment at present except in the production of large lots of virus for the manufacture of vaccine.

1. Original Method of Maitland and Maitland. A fine mince of fowl kidney tissue is prepared by chopping with scissors. Approximately 0 6 ml. of the mince is placed in an Erlenmeyer flask. Medium consisting of 12 ml. of Tyrode's solution and 6 ml of fowl serum is added, and the flask is tightly stoppered. Serial passages of the virus are carried out by introducing either a portion of the medium or a suspension of the ground fragments into freshly prepared cultures.

2. Modifications of the Maitland Technic. This basic procedure has been modified in various ways Modifications that warrant mention here are those introduced by Li and Rivers (1930), Simms and Sanders (1942), Plotz (1937) and Farrell and his co-workers (1953). Li and Rivers found that vaccinia virus multiplies in a medium consisting of tissue fragments suspended only in Tyrode's solution This represents the simplest form of tissue culture which has been devised so far Under these circumstances little or no cellular division occurs, as indicated by the virtual absence of mitosis on histologic examination of the fragments Nevertheless, in this medium chick-embryo cells survive for at least 4 weeks and retain the capacity to multiply when explanted in a complete medium (Enders and Pearson, 1941) Many viruses (Robbins and Enders, 1950), as well as the rickettsiae of typhus and Rocky Mountain fever, have been propagated in the system of Li and Rivers

By adding a few drops of chicken plasma to a mixture of serum, Tyrode's solution and chick-embryo tissue, Plotz (1938) found that the tissue fragments are gathered together in a network of fibrin which tends to float on the surface of the fluid Excellent yields of several viruses can be obtained by this method which permits considerable multiplication of the cells to take place.

Simms and Sanders (1942) introduced the use of an ultrafiltrate of serum instead of whole serum as a constituent of the medium in Maitland cultures Under these conditions they observed multiplication of several viruses The viruses of poliomyelitis and mumps were first cultivated in human non-nervous tissues suspended in a medium of this composition (Enders et al, 1949; Weller and Enders, 1949).

Farrell and his collaborators (1953) adapted the method to the large-scale propagation of poliovirus for the production of

The agar is melted in boiling water and cooled to 43° C The other constituents are brought to this temperature and mixed with the agar.

Hsiung and Melnick (1955) introduced a *simple but very useful modification of* Dulbecco's method. It consists merely of establishing the monolayer and the overlay on one side of a flat-walled bottle which subsequently is tightly stoppered In this way the necessity *of incubation in a humidified incubator in the* presence of CO_2 is eliminated Furthermore, the cells remain viable for a longer period, which permits the development of plaques produced by slowly growing cytopathogenic viruses (e g , Echo and certain Coxsackie viruses) that fail to appear in open Petri dishes (cf Fig. 48).

3 TECHNIC OF WALLACE AND HANKS FOR GROWING CELL COLONIES ON AGAR Wallace and Hanks (1958) have taken advantage of the availability of cell suspensions to produce colonies of cells in which the elements are more closely packed than in living tissues Small loops of a dense cell suspension are placed on agar slants or plates containing appropriate nutriments and incubated at 37° C Arrangements are made for renewal of the liquid phase by slow diffusion Under these conditions L strain mouse fibroblasts form large mucoid colonies during several months *of incubation The effect of viruses and* rickettsiae in cell colonies of this sort has not yet been studied, but it would seem that the technic might be applicable to analysis of host-virus relationships in a single type of cell under conditions more analogous in certain respects to those found in the living animal body than those offered in other types of cell culture

4 TECHNICS FOR THE CULTIVATION OF CELL CLONES A major obstacle to the precise analysis in vitro of viral multiplication as well as of other phenomena involved in virus-cell relationships has been the heterogeneous character of cell populations Primary cultures, whether derived from tissue fragments or [illegible] never can be regarded

geneous, since they originate from the heterogenous population of a primary explant Accordingly, one cannot assume that under these circumstances the behavior of individual cells exposed to virus will be uniform. A far greater uniformity of response not only to viral infection but in all other respects is to be anticipated in the case of a cell clone, i e , a *line derived from a single cell* Not long ago many investigators believed that clonal growth of mammalian cells could not be obtained because repeated experiments seemed to show that multiplication depended upon conditions established by the association of a certain minimal number of cells lying in close proximity Sanford and her collaborators (1948) *demonstrated the fallacy of this hypothesis* when they developed a clone of mouse fibroblasts This was achieved by drawing a single cell into a small capillary tube which was then immersed in a "conditioned medium," i e , medium in which a large population of cells had been grown previously For reasons

FIG 48 *Virus plaques in monolayer* cultures of monkey kidney cells with agar overlay prepared according to Hsiung and Melnick's *modification* (1955) of the technic of Dulbecco and Vogt. The plaques on the right were produced by poliovirus Type III, and those on the left by Echo virus type 8 Note difference in size and outlines of the plaques Approximately natural size (Hsiung, G D , and Melnick, J J Virology *1*, 533)

a vessel which may be of any desired shape or size and then allowing the cells to settle on the glass to which they rapidly become adherent When considerable numbers of replicate cultures in test tubes are required, aliquots of the stock suspension are conveniently dispensed into a series of test tubes by means of an automatic pipette while the cells are kept uniformly dispersed with a magnetic stirrer As routine the cultures are placed at 37° C, in a slightly slanted position and left undisturbed for several days In the pH color test for the assay of viral infectivity or titration of neutralizing antibodies (Salk et al, 1954) the tubes are maintained in a vertical position, allowing the cell layer to form on the bottom This test depends upon the fact that cells affected by the virus cease to metabolize. Accordingly, the pH of the medium remains high in comparison with that in cultures containing living cells which produce large amounts of acid metabolic end products (Robbins et al, 1950) In slanted tubes or in flasks monolayers usually develop after 5 to 10 days and consist of a sheet of contiguous cells as illustrated in Figure 45 At this time or subsequently the viral inoculum usually is introduced, although under certain circumstances, as in the pH color test, it may be added to the suspended cells when the culture is established After addition of the virus, the tubes or flasks are kept stationary, rotated or shaken mechanically. At present the simple monolayer culture is preferred to other types of tissue cultures for the routine propagation of all viruses that can be grown under these conditions.

2. Monolayer Cultures with Agar Overlay. The versatility of the monolayer culture was extended significantly by Dulbecco (1952) With the purpose of developing a technic that could be applied to mammalian viruses comparable with the plaque method of assay for bacteriophage, he covered the bottom of a Petri dish with chick-embryo cells dispersed by mechanical means. When the cells had spread out in the presence of a suitable medium, he exposed them to a dilute suspension of equine encephalomyelitis virus —an agent that was known to destroy rapidly the cells of this species. In order to confine the infection to the immediate neighborhood of the cells that were exposed to each of the few infective virus particles introduced in the inoculum, he covered the entire preparation with a thin overlay consisting of a mixture of agar, nutritive medium and neutral red Following incubation for several days at 37° C, discrete, circular translucent slightly yellowish areas were seen throughout the reddish sheet of cells These macroscopic effects or "plaques" were produced by the cytopathic effect of the progeny of the virus particles developing in the cells originally infected and then spreading, presumably by direct contact, to those adjacent Soon afterward, Dulbecco and Vogt (1954) improved the technic by substituting suspensions of trypsin-dispersed cells for the mechanically disrupted preparations initially employed

Because of the important role it now plays in mammalian virus research, the principal details of the method are presented. A heavy suspension of trypsinized monkey renal or other suitable cells is prepared in Earle's solution, and 2 ml is placed in each of a number of 60-mm pyrex dishes, then 1 5 ml horse serum and 0 75 ml chick embryo extract are added The cultures are incubated in a humidified incubator receiving a continuous flow of 3 per cent CO_2 in air The medium is changed every 3 days until a monolayer containing approximately 2×10^6 cells is formed. This usually requires 3 to 7 days with monkey renal cells Then the monolayer is washed twice with 2 ml of phosphate buffer saline solution After removal of the last washing fluid 0.3 ml of diluted virus suspension is pipetted onto the monolayer. The cultures are placed at 37° C. for 30 minutes to allow adsorption of virus to the cells which are then covered with 3 ml of melted agar overlay at a temperature of 43° C After solidification of the agar at room temperature the cultures are returned to the humidified incubator Under these conditions macroscopically visible plaques are produced by polioviruses types I and II within 24 hours.

The agar overlay consists of:

2.7% agar in distilled H_2O	12 parts
Neutral red in distilled H_2O	12 parts
Earle's salt solution 4x concentrated containing 400 μg of streptomycin and 400 units of penicillin per ml.	8 parts
50% chick embryo extract in Earle's solution	5 parts

Since the feeder-layer technic yielded nearly 100 per cent plating efficiency (as expressed by the ratio of number of single cells growing to number of cells plated), Puck and his associates reasoned that each cell possesses the potentiality for initiating unlimited growth, and that in consequence similar results without a feeder system might be obtained if proper conditions could be found Investigation led to the conclusion that cells were damaged considerably under the standard treatment with trypsin and washing in salt solution used to prepare the suspensions, and in consequence their growth capacity was impaired By taking precautions to minimize exposure to trypsin and salt solution and to reduce mechanical stress occasioned by repeated pipetting, a high plating efficiency was obtained when a few widely separated cells were allowed to settle directly on the bottom of Petri dishes and were supplied with an appropriate growth medium

The details of the method for obtaining clonal growth on glass have been summarized by these authors essentially as follows The medium is removed from a confluent sheet of HeLa cells grown in a bottle or Petri dish The cells are washed gently for a few seconds in a small quantity of balanced salt solution from which the calcium has been omitted The fluid is removed, and an equal volume of 0 05 per cent trypsin in salt solution of the same composition is added After 10 to 15 minutes at 37° C with occasional gentle agitation the action of the enzyme is arrested by addition at room temperature of an equal volume of complete growth medium The latter consists of Waymouth's mixture to which mammalian serum (human or horse, in varying proportions) and Hanks' solution are added The cell suspension is pipetted for the minimal time required to disperse aggregates, and the number of cells is determined in a hemocytometer Any desired aliquot of this suspension (of which 90 per cent or more of the cells are single) is added to a Petri dish (60 mm) containing 4 5 ml of growth medium The culture is incubated at 37° C in an atmosphere of 5 per cent CO_2 for 8 to 9 days Individual cell colonies that develop are removed and subcultured at the end of this time, or they may be stained with hematoxylin or Giemsa after fixation with Bouin's fluid When stained they have the general appearance shown in Figure 49

With the exception of the clonal line of L strain mouse fibroblasts (Sanford et al , 1948), as yet clonal stocks have been little studied in respect to their reactions with viruses or rickettsiae. There is no doubt, however, that in the future they will be regarded as essential in the analysis of certain problems in this area. Already Puck and Marcus (1956) have indicated one important application in experiments in which HeLa cells were exposed to large quantities of Newcastle disease virus The majority of cells were destroyed, but clonal strains were established with the survivors that later were shown to carry the virus for at least 2 years without exhibiting any morphologic abnormality This occult infection, analogous in certain respects to lysogeny in bacteriophage, was revealed by plating out cells from the infected line on feeder layers of x-irradiated HeLa cells The latter proved to be highly susceptible to the cytopathic effect of the virus harbored by the carrier line and were promptly destroyed

Technics of Group II—Suspended Cell Cultures. In the investigation of certain aspects of virus-cell relationships, it has become increasingly apparent that populations of submerged cells growing uniformly throughout a fluid medium would offer ideal materials for the more refined analysis of such fundamental problems as the mechanisms of virus adsorption, penetration and multiplication, the effect of virus on the growth of the cells, and the biochemical and morphologic changes induced in the cell by viral infection From the practical standpoint, systems of this sort would provide a continuous source of virtually unlimited quantities of cells for the routine preparation of monolayer cultures With these considerations in mind several workers during the last 5 years (cf Table 8) have shown that in cultures that are constantly agitated, a number of established cell lines can be propagated as submerged suspensions.

Of the various technics that have been described the most efficient appears to be the so-called spinner culture introduced lately by Cherry and Hull (1956) and by McLimans et al (1957a). To maintain the cells in suspension these authors employed a magnetic

that are not clear, subsequent attempts to obtain clones in this manner often failed.

Through the use of trypsinized cell suspensions Puck and his co-workers (1955a) have been able to overcome these difficulties by two procedures which are termed respectively the "feeder" layer and the glass-plating technic. The former consists in distributing a few dispersed cells over a confluent cell layer composed of the same elements which have been treated previously with x-rays to prevent their subsequent proliferation after the medium is added. The preparation is incubated for several days. During this time active multiplication of many of the unirradiated cells occurs, resulting in the formation of macroscopic colonies (cf. Fig. 49). Then the latter are subcultured in test tubes as clonal lines. In developing this method, Puck and his co-workers employed HeLa cells, which as components of the feeder layer were irradiated with 4,000 to 5,000 r. This dose does not kill these cells or grossly alter their metabolism. The feeder layer thus serves to "condition" the medium, enabling the few scattered unirradiated cells to divide.

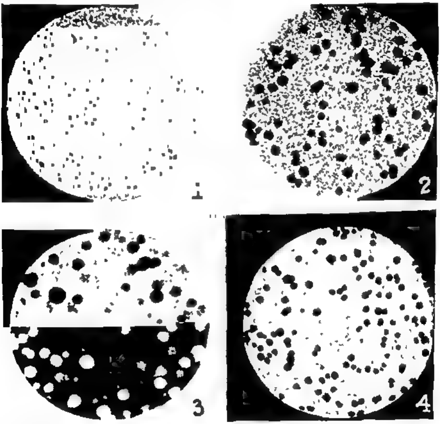

Fig. 49. Demonstration [illegible] for obtaining colonial growth [illegible] ura, 1955). Actual size. (1) [illegible] cells fixed with Bouin's solutic [illegible] (2) Comparable plate with layer of x-irradiated cells to which 100 unirradiated HeLa cells were added 8 days before photograph was taken; 97 colonies were counted. (3) Plate without feeder layer to which 100 HeLa cells [illegible]

countered in the standard aerobic system (Gifford and Syverton, 1957) Although analogous experiments will be necessary with other species of viruses before a final statement can be made, it would seem probable that multiplication will take place in any tension of *oxygen compatible with the viability of the* cells

Hydrogen-Ion Concentration. There is little evidence available to indicate that the fluctuations in hydrogen-ion concentration which ordinarily occur in cultures markedly

characterized by low pathogenicity for monkeys [illegible] delayed in slightly acid media as indicated by the slower formation of plaques in agar overlay cultures of monkey renal cells. The cytopathic changes induced by certain strains of Echo and Coxsackie viruses are also depressed or delayed when the pH of the medium is allowed to become acid (Barron and Karzon, 1957) This "pH effect," which

tions of Echo viruses from fecal specimens may be reduced significantly when acid-producing medium is used Such observations suggest that as routine the pH of the medium should be maintained as nearly as possible within physiologic limits during the cultivation of all mammalian viruses

Composition of the Medium. Numerous investigations have shown clearly that alterations in the composition of the medium may influence markedly the multiplication and the cytopathogenicity of animal viruses For example, Thicke and her co-workers (1952) found that the multiplication of poliovirus continued for a longer time in cultures maintained with medium 199 than in those nourished with Hanks' solution and ox-serum ultrafiltrate, and Scherer (1953) noted that the yield of herpes simplex virus was greater in cells nourished with serum and embryonic extract as compared with those maintained in a serum ultrafiltrate medium In cultures of monkey renal cells Melnick et al (1957) observed that the titer of poliovirus was moderately increased upon addition of cysteine to Hanks' solution Under the same circumstances glycine inhibited the rate of viral multiplication

The large body of data obtained in the past with a variety of agents in Li-Rivers' suspended fragment cultures suggested that their multiplication is not dependent upon the pres*ence of organic compounds, with the possible* exception of glucose. Lately, Melnick et al (1957), working with monkey renal cells, and Eagle and Habel (1956) with HeLa cells have demonstrated that this is true, at least in the case of poliovirus which [illegible] characterized by rapid multiplication in vitro In such instances [illegible] would seem that all organic factors required for viral synthesis except glucose are provided by integral constituents of the cell or its nutrient reserves

In considering the role of substances which appear to be requisite for viral multiplication, such as glucose, it should be borne in mind that this may be quite indirect, consisting perhaps merely in the preservation of cell viability during the period of viral synthesis Therefore, great caution must be exercised in drawing conclusions respecting the biochemical mechanisms involved in replication of virus based upon the effect of variations in the chemical composition of the medium.

That cytopathic changes caused by viruses can also be modified by the presence or the absence of certain nutrients is well illustrated by the observations of Reissig et al (1956) In cultures of human carcinoma cells (strain Hep 2) nourished with a medium consisting of serum, embryonic extract and amniotic fluid these authors noted that the virus of measles induces the formation of syncytia or large giant cells such as described by Enders and Peebles (1954) in cultures of human renal cells When glutamine was added a different sort of change took place, consisting in the assumption of an elongated, fibroblastic-like configuration by individual cells The concentration of glucose in the medium may influence the time at which cytopathic changes appear The presence of normal serum or tissue extracts tends to retard these manifestations of infection As yet only a beginning has been made in the definition of substances that

of this mode of action

stirring rod suspended by a wire attached to a swivel The rod was fixed at a suitable distance from the bottom of the vessel and rotated at a speed that prevented settling of cells but was insufficient to injure them by too violent collision As a further precaution against mechanically induced trauma, 0 2 per cent carboxymethyl cellulose, a substance of high viscosity, was incorporated in the medium which consisted of Eagle's mixture fortified with human, calf or horse serum In this system they demonstrated the continuous growth of L strain fibroblasts, HeLa cells, Chang's conjunctiva cells, human amnion cells and other established strains The capacity of the spinner culture vessels used by McLimans and his associates ranged from 25 to 500 ml From the largest of these 1×10^{6} to 3×10^{8} cells were harvested every 2 or 3 days Recently, McLimans and his collaborators (1957b) have shown that "L" cells can be propagated in commercially available fermentors of 5 liter unit capacity Agitation in the fermentor is accomplished by a mechanism analogous in principle to the spinner culture

FACTORS INFLUENCING INFECTION AND MULTIPLICATION OF VIRUSES IN CELL CULTURES

A number of factors have been defined that influence the multiplication and the cytopathogenicity of viruses in cell cultures, others still unrecognized no doubt exist and will be revealed by future research It has been emphasized already that the natural susceptibility or resistance of cells of different kind and origin is of primary importance In addition, it has been demonstrated repeatedly that variations in the physical, chemical and biologic conditions of the culture profoundly affect the behavior of the virus. Some of these conditions are: temperature of incubation, agitation of the system, oxygen supply, hydrogen-ion concentration and composition of the medium.

Temperature. Although cells in culture may survive for considerable periods when maintained at temperatures varying from about 4° to about 41° C., the range at which most viruses of human origin have been found to multiply is more restricted Little or no multiplication occurs below 20° to 25° C, and toward the upper limit of about 41° C. a slight increase in the temperature of incubation may completely arrest the growth. Thus, Enders and Pearson (1941) noted that influenza A virus (Melbourne strain) fails to multiply in chick-embryo tissue cultures maintained at 40° C., although the cells were not affected appreciably, and Gohd (1958) has shown that no multiplication of this virus (PR8 strain) occurs at 20° C. under similar conditions That the cytopathic effects of viruses may also be modified by changes in temperature is well demonstrated by the experiments of Bang et al (1957), who found that eastern equine encephalitis virus destroyed a strain of rat tumor cells almost completely at 37° C, but that little cytopathogenic effect was seen at 31° C In general, the range 35° to 37° C. employed as routine for the cultivation of mammalian viruses appears to be optimal

Agitation of the Culture System. The favorable influence of continued movement of the medium upon the growth of cells was recognized in the development of the roller-tube culture. That agitation of the system may also increase viral multiplication under certain circumstances is indicated by the observations of Farrell et al (1953), who obtained high yields of poliovirus in suspended fragment cultures of monkey renal cells when these were shaken continuously during the period of viral multiplication Frothingham (1957) has observed in stationary cultures of human amnion cells recently established that little or no proliferation occurs of a chick-embryo adapted strain of type II poliovirus (Roca-Garcia et al, 1952) In contrast a prompt increase accompanied by the appearance of cytopathic changes follows when comparable cultures are rotated With many agents, however, in other culture systems the effect of agitation is not so marked, as illustrated in the experiments of Melnick and Riordan (1952) with poliovirus

Oxygen Supply. Formerly, it was thought that an abundant oxygen supply was essential for optimal viral multiplication, but recently it has been demonstrated that poliovirus can multiply under almost completely anaerobic conditions in HeLa cell cultures and attain concentrations equivalent to those en-

cultures, Ann New York Acad Sc *58*, 1000-1011
Enders, J F., 1954, Cytopathology of virus infections, Ann Rev Microbiol *8*, 473-502
———, 1957, The future of virus studies in tissue culture, J. Nat Cancer Inst *19*, 735-743
Enders, J F, and Florman, A L, 1942, Persistence of vaccinia virus and chick embryonic cells in suspended cell tissue cultures, Proc Soc Exper Biol & Med *49*, 153-155
Enders, J F., and Pearson, H E, 1941, Resistance of chicks to infection with influenza A virus, Proc Soc Exper & Med *48*, 143-146
Enders, J F, and Peebles, T C, 1954, Propagation in tissue cultures of cytopathogenic agents from patients with measles, Proc Soc Exper Biol & Med *86*, 277-286
Enders, J F., Weller, T H, and Robbins, F C, 1949, Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues, Science *109*, 85-87
Evans, V J, Bryant, J C, Fiormonti, M C, McQuilkin, W T, Sanford, K K, and Earle, W R 1956, Studies of nutrient media for tissue cells *in vitro* I A protein-free chemically defined medium for cultivation of strain L cells Cancer Res *16*, 77-86
Farrell, L N, Wood, W, Franklin, A E, Shimada, F T, MacMorine, H G, and Rhodes, A J, 1953 Cultivation of poliomyelitis virus in tissue culture VI Methods for quantity production of poliomyelitis viruses in cultures of monkey kidney Canad J Pub Health *44*, 273-280
Fieldsteel, A H, and Emery, J B, 1954, Cultivation and modification of infectious canine hepatitis virus in roller tube cultures of dog kidney, Proc Soc Exper Biol & Med *86*, 819 823
Fogh, J F., and Lund, R O, 1957, Continuous cultivation of epithelial cell strain (FL) from human amniotic membrane, Proc Soc Exper Biol & Med *94*, 532-537
Frisch, A W., and Jentoft, V, 1953, Use of trypsin in preparing subcultures of monkey testicular tissues, Proc Soc Exper Biol & Med *82*, 322-323
Frisch, A W, Jentoft, V, Barger, R., and Losh, E J, 1955, A human epithelium-like cell (Maben) derived from an adenocarcinoma of lung Isolation, continuous propagation, and effects of selected viruses, Am J Clin Path *25*, 1107-1112
Frothingham, T E, 1957, unpublished data
Gey, G O, 1933, An improved technic for massive tissue culture, Am J Cancer *17*, 752-756
Gey, G O, and Bang, F B, 1951, Viruses and cells—a study in tissue culture applications Tr New York Acad Sc *14*, 15-24
Gey, G O, Coffman, W D, and Kubicek, M T., 1952, Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium, Cancer Res *12*, 264 265
Gey, G O, and Gey, M K, 1936, The maintenance of human normal cells and tumor cells in continuous culture I Preliminary report cultivation of mesoblastic tumors and normal tissues and notes on methods of cultivation Am J Cancer *27*, 45-76
Gifford, G E, and Syverton, J T., 1957, Replication of poliovirus in primate cell cultures maintained under anaerobic conditions, Virology *4*, 141-155
Gohd, R S., 1958, Relationship of temperature to latent influenza virus infection of chorioallantoic membrane in symposium on Latency and Masking in Viral and Rickettsial Infections, p 128, Minneapolis, Burgess
Healy, G M, Fisher, D C., and Parker, R. C, 1955, Nutrition of animal cells in tissue culture X. Synthetic medium no 858 Proc Soc Exper Biol & Med *89*, 71-77
Hsiung, G D, and Melnick, J. L, 1955 Plaque formation with poliomyelitis, Coxsackie and orphan (Echo) viruses in bottle cultures of monkey epithelial cells, Virology *1*, 533-535
Huang, C H, 1942, Titration and neutralization of the western strain of equine encephalomyelitis virus in tissue culture, Proc Soc Exper Biol & Med *51*, 396-398
Jervis, G A, 1955, Comparative susceptibility of tissue culture cells in experimental animals and man, Ann. N Y Acad Sc *61*, 848-851
Lewis, W H, 1935, Rat malignant cells in roller tube cultures and some results Carnegie Institution of Washington Publication 459 Contrib Embryol. *25*, 161-172
Li, C P, and Rivers, T M, 1930 Cultivation of vaccine virus, J Exper Med *52*, 465-470
Lwoff, A, Dulbecco, R, Vogt, M, and Lwoff, M., 1955, Kinetics of the release of poliomyelitis virus from single cells, Ann N Y Acad Sc *61*, 801 805
Lynn, J W, and Morgan, H R, 1954, Cytopathogenicity of animal viruses *in vitro*, A.M.A Arch Path *57*, 301-316
McLimans, W F., Davis, E V, Fletcher, L G, and Rake, G W, 1957a, The submerged culture of mammalian cells the spinner culture, J Immunol *79*, 428-433
McLimans, W F, Giardinello, F E, Davis, E V, Kucera, C J, and Rake, G W., 1957b, Submerged culture of mammalian cells the five liter fermentor, J Bact *74*, 768-774
Maitland H B, and Maitland, M C, 1928, Cultivation of vaccinia virus without tissue culture, Lancet *2*, 596-597
Melnick, J L, 1956, Tissue culture methods for the cultivation of poliomyelitis and other viruses in Diagnostic Procedures for Virus and Rickettsial Diseases, ed 2, pp 97-153, New York, Am Public Health A
Melnick, J L, Hsiung, G D, Rappaport, C., Howes, D, and Reissig, M, 1957, Factors influencing the proliferation of viruses Texas Rep. Biol & Med *15*, 496-533
Melnick, J L., and Riordan, J T, 1952, Poliomyelitis viruses in tissue culture IV Protein-free nutrient media in stationary and roller tub cultures Proc Soc Exper Biol & Med. *81*, 208-213.
Moore, A E, Sabachewsky, L, and Toolan, H W, 1955, Culture characteristics of four permanent lines of human cancer cells, Cancer Res *15*, 598-602

APPLICATIONS

At present there scarcely remains any division of mammalian virology in which the tissue culture method has not found application. Its broad range of usefulness can be compared with that of the broth tube and the agar plate in the study of bacteria and bacteriophages. Indeed, it possesses some advantages not shared by bacterial media, since in certain situations it affords a satisfactory and even superior substitute for the experimental animal. Accordingly, the number of investigations that have depended upon the method as the essential tool is very large, and no attempt will be made to recapitulate here the results of even the most significant

In general, however, the broad applicability of tissue culture can be emphasized by merely listing the many areas of practice and research in which it is now being effectively employed These include

1 Isolation and identification of known viruses

2. Isolation and classification of previously unknown viruses

3 Quantitative determination of viral infectivity

4. Identification and quantitative determination of viral neutralizing, complement-fixing and antihemagglutinating antibodies in the diagnosis of apparent and inapparent infections

5 Development, production, safety-testing and standardization of antisera and vaccines

6. Epidemiologic and ecologic studies

7. Testing of chemical compounds for antiviral activity

8. Studies on the oncolytic properties of viruses

9. Analyses of the physical and chemical properties of viruses

10. Mechanism of viral infection of the cell

11 Mechanism of viral multiplication (kinetics)

12. Problems of natural resistance and susceptibility of cells to viruses

13. Cytopathology of viral infections

14. Variation, mutation and recombination in mammalian viruses

15. Viral interference

16. Mechanism of the reaction between virus and homologous antibody.

References to many advances made in these various areas by the tissue culture method will be found throughout this book. For others the reader is referred to the reviews by Ross and Syverton (1957), Parker (1950), Robbins and Enders (1950), and for the most recent, which have not yet been reviewed, to such publications as *Biological Abstracts.*

REFERENCES

(For complete references, see also 2nd edition, chapter 6)

Bang, F B, Gey, G. O, Foard, M, and Minnegan, D, 1957, Chronic infections produced in cultured cell strains by the virus of eastern equine encephalomyelitis, Virology *4*, 404-417.

Barron, A L, and Karzon, D T, 1957, Effect of pH on cytopathogenicity of orphan viruses, Proc Soc Exper Biol & Med *94*, 393-399.

Berman, L, Stulberg, C S, and Ruddle, F H, 1955, Long term tissue culture of human bone marrow I Report of isolation of a strain of cells resembling epithelial cells from bone marrow of a patient with carcinoma of the lung Blood *10*, 896-911

Bodian, D, 1956, Simplified method of dispersion of monkey kidney cells with trypsin, Virology *2*, 575-576

Carrel, A, 1923, A method for the physiological study of tissues *in vitro*, J Exper Med *38*, 407-418

Chang, R S, 1954, Continuous subcultivation of epithelial-like cells from normal human tissues, Proc Soc Exper Biol & Med *87*, 440-443

Cherry, W, and Hull, R N, 1956, Abstract Tissue Culture Ass meetings, Milwaukee Studies on the growth of mammalian cells in agitated fluid media Anat Rec *124*, 7

Cieciura, S J, Marcus, P I, and Puck, T. T, 1957, The use of x-irradiated HeLa cell giants to detect latent virus in mammalian cells, Virology *3*, 426-428

Dulbecco, R, 1952, Production of plaques in monolayer tissue cultures by single particles of an animal virus, Proc Nat Acad Sc (U S A) *38*, 747-752

Dulbecco, R, and Vogt, M, 1954, Plaque formation and isolation of pure lines with poliomyelitis viruses, J Exper Med *99*, 167-182

Eagle, H, 1955a, Propagation in a fluid medium of a human epidermoid carcinoma, strain KB, Proc Soc Exper Biol & Med *89*, 362-364

———, 1955b, Nutritional needs of mammalian cells in tissue culture, Science *122*, 501-504

Eagle, H, and Habel, K, 1956, The nutritional requirements for the propagation of poliomyelitis virus by the HeLa cell, J Exper Med *104*, 271-287

Earle, W R, 1943, Production of malignancy *in vitro* IV The mouse fibroblast cultures and changes seen in living cells J Nat Cancer Inst *4*, 165-212

Earle, W R., Bryant, J C, and Schilling, E. L., 1954, Certain factors limiting the size of the tissue culture and the development of massive

strain L (Earle) mouse cells, J Nat Cancer Inst *17*, 315-327

Weller, T H, and Enders, J F, 1949, Production of hemagglutinin by mumps and influenza A viruses in suspended cell tissue cultures, Proc Soc Exper Biol & Med *69*, 124-128

Weller, T H, Robbins, F C, and Enders, J F, 1949, Cultivation of poliomyelitis virus in cultures of human foreskin and embryonic tissues, Proc Soc Exper Biol & Med *72*, 153-155

Westwood, J C N, Macpherson, I A, and Titmuss, H H J, 1957, Transformation of normal cells in tissue culture its significance relative to malignancy and virus vaccine production, Brit J Exper Path *38*, 138-154

Younger, J S, 1954, Monolayer tissue cultures I Preparation and standardization of suspensions of trypsin-dispersed monkey kidney cells Proc Soc Exper Biol & Med *85*, 202-205

Zinsser, H, FitzPatrick, F, and Wei, H, 1939, A study of rickettsiae grown on agar tissue cultures, J Exper Med *69*, 179-190

Morann, G L, and Melnick, J L, 1953, Poliomyelitis virus in tissue culture. VI Use of kidney epithelium grown on glass Proc Soc Exper Biol & Med *84*, 558-563

Morgan, J F, Morton, H J, and Parker, R C, *1950*, *Nutrition* of animal cells in tissue culture I Initial studies on a synthetic medium Proc Soc Exper Biol [redacted] Med *73*, 1-8

Murray, M R, and Kopech, G, 1953, Bibliography of the Research in Tissue Culture 1884 to 1950, New York, Acad Press

Owens, O von H, Gey, M K, and Gey, G O, 1954, Growth of cells in agitated fluid medium, Ann New York Acad Sc *58*, 1039-1055

Parker, R C, 1950, Methods of Tissue Culture, ed 2, New York, Hoeber

Plotz, H, 1937, Étude comparative de la virulence des cultures de peste aviaire effectuées en presence de cellules non proliferentes et en présence de cellules en voie de prolifération, Compt rend Soc biol *125*, 603-604

———, 1938, Culture *in vitro* du virus de la rougeole, Bull Acad nat med, Paris, *119*, 598-601

Puck, T T, and Marcus, P I, 1955a, A rapid method for viable cell titration and clone production with HeLa cells in tissue culture the use of x-irradiated cells to supply conditioning factors, Proc Nat Acad Sc (U S A) *41*, 432-437

Puck, T T, Marcus, P I, and Cieciura, S J *1955b*, Clonal growth of mammalian cells *in vitro* Growth characteristics of colonies from single HeLa cells with and without a "feeder" layer J Exper Med *103*, 273-284

Rappaport, C, 1956, Monolayer cultures of trypsinized monkey kidney cells in synthetic medium Application to poliovirus synthesis Proc Soc Exper Biol & Med *91*, 464-470

Reissig, M, Black, F L, and Melnick, J L, 1956, Formation of multinucleated giant cells in measles virus infected cultures deprived of glutamine, Virology *2*, 836-838

Robbins, F. C, and Enders, J F, 1950, Tissue culture techniques in the study of animal viruses, Am J M Sc. *220*, 316-338

Robbins, F C, Enders, J F, and Weller, T H, 1950, Cytopathogenic effect of poliomyelitis viruses *in vitro* on human embryonic tissues, Proc Soc Exper Biol & Med *75*, 370-374

Roca-Garcia, M, Mayer, A W, and Cox, H R, 1952, Poliomyelitis II Propagation of MEF_1 strain of poliomyelitis virus in developing chick embryo by yolk sac inoculation Proc Soc Exper Biol & Med *81*, 519-525.

Ross, J D., and Syverton, J T, 1957, Use of tissue cultures in virus research, Ann Rev Microbiol *11*, 459-508.

Rous, P, and Jones, F. S, 1916, A method for obtaining suspensions of living cells from the fixed tissues, and for the plating out of individual cells, J. Exper. Med *23*, 549-555

Salk, J., and Ward, E N, 1957, Some characteristics of a continuously propagating cell derived from monkey heart tissue, Science *126*, 1338-1339

Salk, J E, Younger, J S, and Ward, E N, 1954, Use of color change af phenol red as the indicator in titrating poliomyelitis virus or its antibody in a tissue culture system, Am J. Hyg. *60*, 214-230

Sanders, M, Kiem, I, and Lagunoff, D, 1953, Cultivation of viruses, A.M.A Arch Path *56*, 148-225

Sanford, [redacted] K, Earle, W R, and Likely, G D, 1948, The growth *in vitro* of single isolated tissue cells, J Nat Cancer Inst *9*, 229-246

Scherer, W F, 1953, The utilization of a pure strain of mammalian cells for the cultivation of viruses *in vitro*, Am J Path *29*, 113-137

Scherer, W F, and Hoogasian, A C, 1954, Preservation at subzero temperatures of mouse fibroblasts (strain L) and human epithelial cells (strain HeLa), Proc Soc Exper Biol & Med *87*, 480-487

Scherer, W F, and Syverton, J T, 1954a, The viral range *in vitro* of a malignant human epithelial cell (strain HeLa, Gey) I Multiplication of herpes simplex, pseudorabies and vaccinia viruses Am J Path *30*, 1057-1073

———, 1954b, The viral range *in vitro* of a malignant human epithelial cell (strain HeLa, Gey) I Multiplication of herpes simplex, pseudorabies and vaccinia viruses Am J Path *30*, 1075-1083

———, 1955, The viral range *in vitro* of a malignant human epithelial cell (strain HeLa, Gey) III Studies with pseudolymphocytic choriomeningitis virus General discussion, Am J Path *31*, 31-39

Scherer, W. F, Syverton, J T, and Gey, G O, 1953, Studies on the propagation *in vitro* of poliomyelitis viruses IV Viral multiplication in a stable strain of human malignant epithelial cells (strain HeLa) derived from an epidermoid carcinoma of the cervix J Exper Med *97*, 695-710

Sheffield, F W, and Churcher, G, 1957, The serial propagation of poliomyelitis viruses in cells derived from rabbit embryo kidney, Brit J Exper Path *38*, 155-159

Simms, H S, and Sanders, M, 1942 Use of serum ultrafiltrate in tissue cultures for study of deposition of fat and for propagation of viruses, Arch Path 33, 294-300

Stulberg, C S, and Schapira, R, 1953, Virus growth in tissue culture fibroblasts I Influenza A and herpes simplex viruses J Immunol *70*, 51-59

Thicke, J C, Ducam, D, Wood, W, Franklin, A E, and Rhodes, A J, 1952, Cultivation of poliomyelitis virus in tissue culture I Growth of the Lansing strain in human embryonic tissues Canad J M Sc *30*, 231-245

Topacio, T, and Hyde, R R, 1932, The behavior of rabbit virus III in tissue culture Am J Hyg *15*, 99-124

Vogt, M, Dulbecco, R, and Wenner, H A, 1957, Mutants of poliomyelitis viruses with reduced efficiency of plating in acid medium and reduced neuropathogenicity, Virology *4*, 141-155

Wallace, J H, and Hanks, J. H, 1958, Personal communication to the author

Waymouth, C., 1956, A serum-free nutrient solution sustaining rapid and continuous proliferation of

strain L (Earle) mouse cells, *J Nat Cancer Inst* *17*, 315-327
Weller, T H, and Enders, J F, 1949, Production of hemagglutinin by mumps and influenza A viruses in suspended cell tissue cultures, Proc Soc Exper Biol & Med *69*, 124-128
Weller, T H, Robbins, F C, and Enders J F, 1949, Cultivation of poliomyelitis virus in cultures of human foreskin and embryonic tissues Proc Soc Exper Biol & Med *72*, 153-155
Westwood, J C N, Macpherson, I A, and Titmuss, H H J., 1957, Transformation of normal cells in tissue culture its significance relative to malignancy and virus vaccine production, Brit J Exper Path *38*, 138 154
Younger, J S, 1954, Monolayer tissue cultures I Preparation and standardization of suspensions of trypsin-dispersed monkey kidney cells Proc Soc Exper Biol & Med *85*, 202-205
Zinsser, H, FitzPatrick, F, and Wei, H., 1939, A study of rickettsiae grown on agar tissue cultures, J Exper Med *69*, 179-190

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10

Serologic Reactions in Viral and Rickettsial Infections

INTRODUCTION

The several serologic procedures constitute an important basic methodology for the study of viral and rickettsial diseases and of their causal agents The importance of serologic methods is shown by the fact that the greatest proportion of examinations conducted in a diagnostic laboratory consists of serologic tests (Lennette, 1956); that epidemiologic investigations depend, to a major degree, upon serologic methods; and that such methods form an integral part of any approach to the study of the properties and the nature of viral and rickettsial agents, even in what are purely biochemical or biophysical investigations. Most of the methods represent adaptations of the basic bacteriologic or immunologic technics, the modifications having been necessitated through the inescapable use of crude, or weak, antigens or both In recent years, however, considerable progress has been made in freeing viral antigens of extraneous materials and in increasing their potency and sensitivity in in vitro tests.

Each of the several serologic tests is still performed in a greater or lesser variety of ways. As mentioned, some modifications were required by the relative impurity and insensitivity of the available antigens; some of the modifications, however, are referable to personal preference based on individual experience It is not possible here, nor desirable, to present in detail each of the various tests employed for each viral and rickettsial disease discussed in this book It is intended, rather, to present a general consideration of each of the basic immunologic procedures; the student interested in detailed descriptions of serologic procedures and methodologies is referred to the excellent compendium on technical procedures issued by the American Public Health Association's Committee on Diagnostic Procedures for Virus and Rickettsial Diseases (1956).

NEUTRALIZATION

The neutralization test is based upon the fact, dating from Sternberg's original observation with vaccine virus in 1892, that infection, overt or inapparent, with a viral agent leads to the development of a humoral antibody capable of neutralizing, or nullifying, the infective capacity of the agent. The test was used for many years before less complex but equally specific in vitro methods were developed and is still the standard against which the specificity of other methods is evaluated Neutralizing antibody, which is highly specific and generally accepted as the antibody responsible for the protective effect of im-

mune serum, is detected and assayed by inoculation of suitably prepared serum-virus mixtures into a host system that will serve as an indicator of the presence or the absence of active virus in the mixture, i e, whether neutralization of infectivity has or has not been achieved in the mixtures Since demonstration of infectivity requires the use of living tissues, the host systems employed are various animal species, but especially the albino Swiss mouse, the embryonated hen's egg, and mammalian or avian cell cultures

The neutralization technic is still employed comparatively widely Indeed, in some situations it may be the method of choice and, in others, the only serologic technic available. Thus, while the method may be used diagnostically to detect the appearance, or rise in titer, of neutralizing antibody during the course of a patient's illness or for the identification of a viral agent isolated from a patient, often the same objective can be reached by other, simpler procedures On the other hand, since neutralizing antibody may persist over many years, whereas the persistence of complement-fixing antibody, for example, is of relatively short duration, the neutralization test usually provides the only means to measure the extent to which a population has been immunized to a given virus Similarly, since artificial active immunization may lead to the development of little or no complement-fixing antibody, reliance must be placed upon the neutralization test, when other, simpler methods are lacking, to determine the immunogenic potency of the vaccine As is evident from the chapters dealing with specific diseases, the neutralization test, while highly valuable in virology, has found little application to the problems of the rickettsiologist

Technics for performing neutralization tests vary widely The emergence of certain modifications which stem from differences in host indicator systems might be expected Similarly, differences between agents may require the use of animal hosts of different ages or necessitate inoculation of the serum-virus mixtures by different routes Other differences in methodology, such as differences in the ratio of virus-to-serum volumes employed, incubation or nonincubation of serum-virus mixtures, differences in the time and the temperature of incubation, when this is employed, etc, indicate that a single method has not been found satisfactory for use with all viral agents, nor for the specific purposes to which the test is to be applied Despite the diversity of technics, the basic principle on which all the procedures are based is the same

Before outlining the framework on which neutralization tests are built, a few words are pertinent with respect to the measuring tools used to ascertain the infectivity of viral preparations and to determine the neutralizing capacity of immune serum Quantitation in virology involves the testing of a series of dilutions (of virus, of immune serum, etc) and the endpoint is generally represented by that dilution at which a certain proportion of the test animals reacts or dies A 100 per cent endpoint titer is unacceptable because its accuracy is affected so greatly by minor chance variations The most desirable type of endpoint is one based on an assay in which one half of the animals react, and the other one half do not Such an endpoint can be determined experimentally, but to do so requires the use of large numbers of test animals at closely spaced dilutions near the value for 50 per cent reaction Aside from other objections, the cost of the large number of animals used on every dilution in itself virtually precludes such a method for quantitation To circumvent such difficulties, Karber (1931) and Reed and Muench (1938) devised simpler methods for the estimation of 50 per cent endpoints The methods are described, and their use illustrated, in an article (Lennette, 1956) to which the interested student is referred As mentioned earlier, a series of dilutions of the test material to be assayed is made and inoculated into groups of animals, e g, 6 to 8 mice per dilution In such an assay, a large number of animals is used, and the 50 *per cent endpoint titer estimations are based* on the large *total* number of animals involved Computations based on the total number of animals used in the titration gives the effect "of using, at the two critical dilutions between which the endpoint lies, larger groups of animals than were actually included at these dilutions By inclining to equalize chance variations, the method tends to define the point more nearly than would be possible if it were simply interpolated between the two bracketing results" (Reed and Muench,

1938) The 50 per cent endpoint can be based on several types of reactions The most widely used endpoint in animal host systems is based on *mortality* and a written LD_{50} (50% lethal dose). The dose which *infects* 50 per cent of the test animals is written ID_{50}, the dose which *paralyzes* 50 per cent of the animals is represented by PD_{50}, etc With tissue culture systems, the term TCD_{50} or $TCID_{50}$ represents that dose of virus which gives rise to cytopathogenic changes or to colorimetric changes in 50 per cent of the inoculated cultures

The virus used in neutralization tests consists of a stock suspension whose infectivity or lethality endpoint is accurately known from replicate titrations previously done; knowledge of the endpoint permits preparation of the appropriate dilution or range of dilutions of the stock suspension to provide the requisite amounts of virus to be used in the examination of the serum The neutralizing capacity of a serum can be ascertained in either of two ways, namely, by the varying virus-constant serum method or by the constant virus-varying serum method The varying virus-constant serum method [illegible] used [illegible] als as [illegible] od, a series of virus dilutions is selected so that the control titration will cover the range of 100 per cent mortality at one end and 100 per cent survival at the other. The extent to which the infectivity of the virus is reduced by the test serum is a measure of the neutralizing capacity of the latter. Thus, in testing paired or multiple serum specimens obtained from a patient during the acute and the recovery or convalescent stages of an illness, the amount by which the recovery or convalescent phase serum specimen reduces the infectivity of the virus is compared with the amount of reduction in infectivity effected by the acute phase serum, which serves as the base line. Thus, the titer of the virus mixed with the acute phase serum may be 1×10^{-8}, whereas the titer of the virus mixed with the convalescent phase serum may be $1 \times 10^{-5.5}$; the difference between these two endpoint titers is $10^{2.5}$ or, arithmetically, approximately 300. This difference between the endpoint titers of the virus in the presence of the two sera is referred to as a "neutralization index" In this instance, the neutralization index is 300 Expressed in other terms, the neutralization index indicates the number of 50 per cent endpoint doses of virus, for example LD_{50}, that is neutralized by the convalescent phase serum as compared with the acute phase serum. In general, a neutralization index of 1 to 9 is regarded as negative, of 10 to 49 as equivocal, and of 50 or more as positive. Consequently, a diagnostically significant rise in neutralizing antibody titer is one in which the neutralization index, based on the comparison of acute and convalescent phase serums, is 50 or greater (log 1.7). In epidemiologic surveys, where only a single serum specimen from an individual is tested, the same criterion applies, i e, a positive serum is one capable of neutralizing 50 or more LD_{50} of virus.

With some viruses, the embryonated hen's egg can be used as a test species for neutralization tests. Most *in ovo* technics utilize the varying virus-constant serum method and, as in the case of animal tests, several types of endpoints may be used to express the neutralizing capacity of a serum. The first type of endpoint can be used with those viruses which produce plaques, or pocklike lesions, on the chorio-allantoic membrane Serum-virus mixtures representing a series of virus concentrations added to undiluted test serum are inoculated into embryonated hens' eggs. The degree of neutralization effected by the serum is reflected by the quantitative reduction in the number of virus particles capable of producing lesions, as indicated by the number of plaques produced. The procedure is time-consuming and open to a number of errors; reading and interpretation of the findings requires considerable experience. Replicate titrations of the infectivity of the virus alone are apt to vary markedly; when serum-virus mixtures are titrated, a further error arises from the lack of proportionality between virus dilutions and plaque counts (Jawetz and Coleman, 1952). A more nearly accurate endpoint for the quantitative measurement of antibody is that based on the lethal action of free virus in the mixtures; even so, the method has not found very wide acceptance in diagnostic virology, perhaps because most of the diseases with causal viruses which produce lethal effects in the chick embryo can be diagnosed by the much simpler in vitro serologic methods The third endpoint is feasible only with certain viruses, since it depends upon the appearance or the nonappearance in individual eggs of hemagglutinins or complement-fixing antigens, depending upon whether or not free virus is present in the serum-virus mixture. Determination of

such an endpoint is obviously much more cumbersome than is the determination of one based upon mortality.

With certain other viruses, quantitation of neutralizing antibody can be done in tissue culture systems A variety of tissues, both animal and human, and including human cancerous tissue, has been used In general, the sera are tested by the constant virus-varying serum method Basically, there are 3 different methods for conducting neutralization tests in tissue culture systems, viz, in monolayer cell cultures in tubes, in suspensions of dispersed cells, or by the plaque technic of Dulbecco and Vogt (1954a, 1954b) In the monolayer cell method (e g, monkey kidney epithelial cells, or strain HeLa human carcinoma cells), serial dilutions of serum, usually in 2-fold decrements, are tested against a standard dose of virus, usually 100 TCD_{50} as determined from prior titrations of the stock virus Each serum-virus mixture is inoculated into a group of several culture tubes The cultures are held in an incubator for several days and at intervals are examined microscopically Mixtures in which the virus is completely neutralized have no effect on cell growth, and the cell sheets appear to be normal Mixtures which contain free or unneutralized virus give rise to cytopathogenic changes in the cultures A 50 per cent endpoint for the neutralizing capacity of the serum can be computed, using the presence or the absence of cytopathogenic changes in the culture tubes as the basis for estimation

The second type of test, and one especially applicable to large-scale use, is performed with suspensions of susceptible cells This technic, generally referred to as the colorimetric test, the pH color test, or the metabolic-inhibition test, was first described by Salk et al (1954) for use with monkey kidney epithelial cells, a similar technic for use with HeLa cells was described by Lipton and Steigman (1955) and Robertson et al (1955) In this test, dilutions of serum are tested against standard doses of viruses, usually 100 TCD_{50} The serum-virus mixtures may be tested either in small tubes or in disposable plastic panels with indentations or cups to hold the mixtures To each serum-virus mixture is added a standard aliquot of cell suspension appropriately diluted to contain the desired number of cells in the aliquot used This test eliminates the need for cells which have grown out in a monolayer on glass The test is based on the fact that changes in the color of the phenol red indicator, i e, changes in pH level, reflect the metabolic activity of the cells Phenol red has a deep red color at pH 7 4 and above, but becomes salmon-colored, and finally yellow, as the pH drops to 7 2 and below Unneutralized virus present in a serum-virus mixture infects and kills the cells, since cellular metabolism is halted, the medium remains red Conversely, cells in the presence of a completely neutral serum-virus mixture are able to continue their metabolic activity with a resultant accumulation of acidic materials and a lowering of pH

The plaque technic of Dulbecco and Vogt (1954a, 1954b) can be used for the quantitation of neutralizing antibody (Dulbecco et al, 1956) This technic is based on the fact that certain viruses when mixed with a suspension of dispersed susceptible cells and then plated out give rise to plaques, or colonies, in a fashion analogous to the plaques obtained with bacteriophage The neutralizing capacity of the serum is indicated by the degree to which it can reduce plaque formation by the virus The technic permits highly accurate titrations of virus as well as very accurate determinations of antibody levels The method is most useful in basic immunologic and virologic studies, for ordinary diagnostic work and epidemiologic studies, it does not have the simplicity of the colorimetric method.

Neutralization of infectivity is not a property unique to specific antibody, since normal human and animal sera have been found to possess, in varying degree, the ability to effect nonspecific neutralization of certain viruses, e g, influenza, mumps, and Newcastle disease (Ginsberg and Horsfall, 1949) This nonspecific virus inactivating substance is heat-labile and storage-labile, it can be destroyed by heating at 56° C for 30 minutes or by simple storage of the sera at the usual icebox temperatures of 4° to 6° C. The role of this nonspecific inactivator in the neutralization reaction, and whether it should be preserved or eliminated from sera which are to be examined, is a debatable point Since this nonspecific factor can neutralize not inconsiderable amounts of virus, the use of unheated sera in neutralization tests would appear to be a hazardous procedure (Gins-

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generally, but not invariably, possess activity. How enhancement of the viral content of a tissue, with a concomitant increase or appearance of complement-fixing antigen, can be effected by substitution of a more susceptible cell for a less susceptible one, is exemplified by dengue virus. When this virus is propagated in the infant mouse brain, it attains much higher infectivity levels than it does in the adult mouse brain, this marked increase in concentration is accompanied by the appearance of practical amounts of complement-fixing antigen in the tissue. Similarly, the yield of complement-fixing antigen can be increased by increasing the number of susceptible cells available for viral synthesis, as has been done for poliomyelitis viruses in tissue culture (Schmidt et al., 1957). Lastly, the development of suitable antigens has also been based upon the ability to achieve some purification of crude tissue suspensions through the removal of undesirable extraneous materials.

Crude tissue suspensions, although they may be rich in viral content and in the products of viral growth, are generally unsatisfactory as antigens from one or more standpoints; this is especially true of CNS tissue suspensions containing any one of a number of neurotropic viruses. The crude brain tissue suspensions tend to be anticomplementary in variable degree, but even when free of such reactivity they frequently show a tendency to fix complement in the presence of normal sera. This phenomenon has been attributed, in part, to the presence in normal serum of a natural antibody which reacts with some constituent of normal tissue (Kidd and Friedewald, 1942); this natural antibody can be destroyed by heating at temperatures (60° to 65° C.) higher than that generally used for inactivation (56° C.), and the reacting substance in the antigen can be removed by high-speed centrifugation. Maltaner's work (1946) suggested that the reaction is entirely nonspecific in nature and due to a cephalinlike substance present in tissue extracts; he showed that many tissue suspensions used as antigens possess thromboplastic activity, that inactivated human serum enhances the thromboplastic activity of such antigens, and that there is a direct correlation between thromboplastic activity and the occurrence of nonspecific fixation. In addition to this tendency to react with normal sera, crude antigens also have a propensity to give fixation with Wassermann positive sera. Lastly, crude tissue antigens, if not anticomplementary initially, tend to become so with storage.

The removal of substances responsible for nonspecific and anticomplementary reactivities is based essentially on 3 different approaches, viz., (1) high-speed centrifugation, (2) repeated freezing and thawing and (3) extraction with lipid solvents. The conclusion of Kidd and Friedewald (1942) that a sedimentable constituent of normal tissue is involved in nonspecific reactions was substantiated by Havens et al. (1943); the latter workers found that antigens free of the sedimentable fraction could be used with sera heated at 56° C. and that higher inactivating temperatures were not required. Experience in the author's laboratory with chick-embryo-derived antigens indicates that nonspecifically reactive substance can be largely, but not entirely, sedimented by high-speed centrifugation; the purified antigens occasionally react with normal serum, although the incidence of such nonspecific reactions is markedly less as compared with crude antigen preparations (Lennette et al., 1956).

Casals and Palacios (1941) noted that high-speed centrifugation, especially if followed by Seitz filtration, served to remove much of the nonspecific and anticomplementary activity of infected mouse-brain suspensions. Because filtration was accompanied by reduction in antigenicity, they preferred to subject their crude tissue emulsions to repeated freezing and thawing which served to flocculate nonspecifically reactive materials; however, the use of antigens purified by this procedure required inactivation of the test sera at 60° to 65° C., depending upon the animal species of origin of the serum.

At the present time, antigens for the neurotropic virus diseases are prepared from infected mouse-brain suspensions extracted with lipid solvents to remove nonspecifically reactive substances. Although earlier workers had used lipid solvents for the purification of precipitating antigens (Craigie and Tulloch, 1931) and complement-fixing antigens (Howitt, 1937), the method was not utilized widely until its reintroduction by DeBoer and Cox (1947). The method of the latter workers was simplified and considerably shortened, in

Karzon's studies (1956) on the nature of the nonspecific virus inactivator present in human and mammalian sera suggest that the nonspecific neutralizing factor active against Newcastle disease virus is related to properdin. The investigations of Wedgwood et al (1956) indicate that the properdin system is probably identical with the inhibitors which have been described for the mumps, influenza A and B, and Newcastle disease viruses, and that it can inactivate these viruses in the absence of antibody The fact that specific neutralizing capacity which has been lost by storage or heating of the serum can be restored entirely, or in major part, by the addition of fresh normal serum, points to the existence of a potentiating factor in human and mammalian sera This potentiating factor may also be identical with the properdin system, since the participation of specific antibody in the neutralization reaction does not preclude the participation of the properdin system—both may act synergistically (Wedgwood et al, 1956)

What processes might be involved in the neutralization of viruses by specific immune sera has always posed a question of considerable interest and one not entirely answered as yet. The observation of Andrewes (1928) that noninfectious mixtures of vaccine virus and immune serum, or virus III and immune serum, became infective on dilution, i e, were reactivated, was confirmed subsequently by a number of investigators working with a variety of viruses These findings led to the opinion that no in vitro union of virus and neutralizing antibody occurs, and that the reactivation phenomenon is peculiar to viruses Craigie (1939) evaluated the available evidence and concluded that both ideas were erroneous, i e., union of virus and antibody does occur in vitro and reactivation of neutral mixtures does not occur. The most recent studies on the kinetics of the neutralization test are those of Dulbecco et al (1956), who used the western equine encephalomyelitis and type 1 poliomyelitis viruses as models, and the plaque method of assay which, as mentioned above, is a highly accurate method for the quantitation of virus and of antibody It was found that neutralization proceeded with time as a first order reaction; its rate was proportional to the concentration of antibody present and was affected by temperature No reactivation (poliomyelitis virus) or only a slight reactivation (western equine encephalomyelitis virus) occurred when serum-virus mixtures were diluted. This lack of reactivation is contrary to experiences previously reported, as mentioned above It seems likely that the reactivation reported by many previous investigators may have been a spurious phenomenon resulting from inadequacies of the assay method which permitted free antibody and virus to be carried over into the quantitating system (susceptible host) with consequent further possible interaction between the two. However, some reactivation of the western equine encephalomyelitis virus could be effected if a high titered virus preparation were added to the virus-serum mixture; either active or ultraviolet light inactivated virus could be used to bring about reactivation Such reactivation is thought to arise from the collision of free viral particles with the virus-antibody complexes

COMPLEMENT FIXATION

The simplicity of the complement-fixation test and the rapidity with which results can be obtained led many earlier workers to attempt application of the method to virologic problems These attempts were, on the whole, either negative or equivocal (Sosa-Martinez and Lennette, 1955) Much of the early difficulty was referable to the crudeness of the antigens available at that time. Over the past 10 to 15 years, however, as antigens of adequate potency and sensitivity have been developed, the method has become an increasingly important one in both theoretical and applied virology. Although the test may be performed with a number of variations in procedural detail (Lennette, 1956), the immunologic principles on which it is based are identical, whether viral, rickettsial, bacterial or other types of antigens are employed.

The development of suitable antigens has rested on several considerations. Generally speaking, the complement-fixing activity of an infected tissue is roughly related to its viral content as measured by infectivity, i e., tissues with a viral content below a certain infectivity level may possess no demonstrable complement-fixing capacity, whereas tissues with a viral content above that critical level

sist, are difficult to make. Thus, in mumps and influenza, complement-fixing antibody appears early, i e , during the first week or 10 days after onset, whereas in poliomyelitis, complement-fixing antibody appears late, i e , frequently during the third or fourth week after onset of symptoms. In the first two diseases, frequently a diagnosis can be made on the mere presence in the acute-phase serum of appreciable amounts of antibody to the S (soluble) complement-fixing antigen, whereas in poliomyelitis, the existence of S (soluble) and V (viral) antigens remains to be ascertained definitely. In most neurotropic viral diseases, complement-fixing antibodies appear later than do neutralizing antibodies; an exception is lymphocytic choriomeningitis, in which the reverse occurs As to the maximal titers attained, levels of 1 128 and higher are not exceptional in the rickettsial and the pneumotropic viral diseases and in some neurotropic

somewhat longer in the rickettsial diseases In some instances, immunization leads to the appearance of complement-fixing antibody, in others it does not Information on which situation obtains, in any given instance, is basic to laboratory diagnosis, which may have to rest upon the ability of the virologist to distinguish between antibody engendered by immunization and that elicited by infection

The complement-fixation test can sometimes be applied to the rapid identification of newly isolated viruses Smadel and Wall (1941) used the test for the rapid identification of lymphocytic choriomeningitis virus in animals inoculated with clinical material from patients, Hummeler et al (1952) used it for the identification of newly isolated influenza viruses, and Lennette et al (1956b) used it for the identification of western equine encephalomyelitis virus in embryonated hens' eggs inoculated with suspensions of infected mosquitoes In addition to its usefulness in the identification of viruses, the test has been applied recently to the typing of viruses which exist in a number of immunologic groups, for example the adenovirus group (Pereira, 1956) and poliomyelitis viruses (LeBouvier et al , 1954, Schmidt and Lennette, 1957)

The sera of certain avian and mammalian species exert an inhibitory effect in specific complement-fixation systems (Rice, 1948), and consequently an indirect method for detecting the occurrence of fixation has to be employed The indirect complement-fixation technic is widely employed for the detection of Newcastle disease antibodies (Wolfe et al , 1949) in the sera of fowl, and for the detection of specific antibody in the sera of chickens, ducks, turkeys, pheasants and other avian species infected with ornithosis virus (Karrer et al , 1950; Hilleman et al , 1951) The inhibitory antibody lacks the ability to combine with guinea pig complement, although it has the capacity to combine with

infected pigeons or humans, or from immunized animals, such as the rabbit If, in an avian serum-ornithosis antigen mixture, the inhibiting antibody unites with the antigen, no antigen is available to combine with the specific indicator antibody and the guinea pig complement, hence, hemolysis of the red cells in the hemolytic system occurs If the avian serum lacks antibodies, the indicator antibody fixes the complement in the presence of specific antigen, no free complement is available, and hence no hemolysis occurs when the hemolytic system is added

The conglutinating complement absorption test (CCAT) represents another modification of the complement-fixation technic and has

lysis The test system consists of dilutions of the serum under examination, antigen and nonhemolytic complement (cat or horse serum) After appropriate incubation of the mixture, the conglutinating system is added,

hemagglutinin present in fresh bovine serum which reacts with a sensitized cell in the presence of nonhemolytic complement. If the test serum contains antibody, complement is

terms of the time required for the processing procedure, by España and Hammon (1948), but it is potentially hazardous since it involves extraction of a highly infectious powder *obtained by lyophilization of the virus suspension* More recently, Casals (1949) has described the preparation of acetone-ether extracted antigens The *advantages of this* method lie in the fact that highly specific antigens can be produced in less than one day, and no special equipment is necessary For details of this method the reader is referred to *the original publication (Casals, 1949)*

Many of the neurotropic viruses possess some degree of viscerotropism, but the affinity for visceral tissue usually is of so relatively low an order that both the virus content and the complement-fixing antigen content of the affected organs is low. One outstanding exception is lymphocytic choriomeningitis virus, the spleens of guinea pigs infected with this virus are a comparatively rich source of specific antigen (Smadel et al, 1939) Similarly, while many neurotropic viruses can be propagated in the embryonated hen's egg, the growth of only a few of them is accompanied by the appearance of satisfactory amounts of complement-fixing antigen in the membranes or the fluids, mention might be made of Murray Valley encephalitis virus (French, 1952), lymphocytic choriomeningitis virus (Whitney et al., 1953), herpes simplex virus (Sosa-Martinez and Lennette, 1955), and western equine encephalomyelitis virus (Lennette et al., 1956). It seems likely that infected tissue culture fluids will serve as the starting material for the preparation of complement-fixing antigens for an increasing number of viral agents. Fluids as antigens are already used in several instances, e.g, the adenovirus group (Hilleman and Werner 1954. Huebner et al, 1954) and, among the neurotropic agents, poliomyelitis viruses (Schmidt and Lennette, 1955) At the present time, however, infected brain tissue is still the chief starting material for the preparation of complement-fixing antigens for neurotropic viruses

Highly satisfactory complement-fixing antigens for the diagnosis of the common rickettsial diseases *can be prepared from the appropriate* rickettsial agent by any of several technics, all of which are based on extraction of infected tissue suspensions with lipid solvents (Smadel *in* American Public Health Association Committee, 1956; Topping and Shepard, 1946). Depending upon the procedure employed, either type-specific or group-specific *antigens can be prepared. Type-specific antigens*, which usually permit differentiation of closely related rickettsial infections, are *indispensable in those situations where it is* highly important to determine with exactitude the rickettsial agent responsible for illness in a given patient, or for a given outbreak Group-specific antigens, on the other hand, *are adequate for most diagnostic purposes, as they serve to differentiate infections* caused by the epidemic typhus-murine typhus group from those caused by other rickettsiae The reader interested in methods for preparing rickettsial antigens is referred to the excellent description of Smadel (*American Public Health Association Committee, 1956*).

Detailed descriptions of the complement-fixation test as applied to the diagnosis of viral and rickettsial diseases are given elsewhere (see various chapters in American Public Health Association Committee, *1956*). Additional description of this comparatively well-known method is not warranted here, and the reader desiring details of methodology is referred to the sources just mentioned. The method employed in the author's laboratory, and standardized for use with a number of viral and rickettsial diagnostic antigens, is also described in those sources (see Jensen *in* American Public Health Association Committee, 1956).

Application of the complement-fixation test in the diagnosis of viral and rickettsial infections necessitates not only a thorough familiarity with the technic, its advantages, disadvantages and inherent pitfalls but also some familiarity with the clinical features of specific infections and, ideally, some knowledge of the epidemiologic characteristics of the various disease entities The same desiderata hold true for other tests in the diagnostic armamentarium but are emphasized here because *of the increasingly wider application that* is being made of the complement-fixation method

Generalizations as to the time of appearance of complement-fixing antibodies, the maximal titers that are attained, and the length of time over which such antibodies per-

after onset, whereas in poliomyelitis, complement-fixing antibody appears late, i e, frequently during the third or fourth week after onset of symptoms *In the first two diseases,* frequently a diagnosis can be made on the mere presence in the acute-phase serum of an-

Y (viral) antigens remains to be ascertained definitely. *In most neurotropic viral diseases,* complement-fixing antibodies appear later than do neutralizing antibodies; an exception is lymphocytic choriomeningitis, in which the reverse occurs As to the maximal titers attained, levels of 1:128 and higher are not exceptional in the rickettsial and the pneumotropic viral diseases and in some neurotropic viral diseases, although in the latter group, as

infection with a neurotropic virus but persist somewhat longer in the rickettsial diseases In some instances, immunization leads to the appearance of complement-fixing antibody, in *others it does* not *Information on which situation* obtains, in any given instance, is basic to laboratory diagnosis, which may have to rest upon the ability of the virologist to distinguish between antibody engendered by immunization and that elicited by infection

The *complement-fixation* test can sometimes be applied to the rapid identification of newly isolated viruses Smadel and Wall (1941) used the test for the rapid identification of lymphocytic choriomeningitis virus in animals inoculated with clinical material from patients, Hummeler et al (1952) used it for the identification of newly isolated influenza viruses, and Lennette et al (1956b) used it for the identification *of western equine* encephalomyelitis virus in embryonated hens' eggs inoculated with suspensions of infected mosquitoes *In addition to its usefulness in the identification* of viruses, the test has been applied recently to the typing of viruses which exist in a number of immunologic groups, for example the adenovirus group (Pereira, 1956) and poliomyelitis viruses (LeBouvier et al, 1954; Schmidt and Lennette, 1957)

The sera of certain avian and mammalian species exert an inhibitory effect in specific complement-fixation systems (Rice, 1948), and consequently an indirect method for detecting the occurrence of fixation has to be employed The indirect complement-fixation technic is *widely employed* for the detection of Newcastle disease antibodies (Wolfe et al, 1949) in the sera of fowl, and for the detection of specific antibody in the sera of chickens, ducks, turkeys, pheasants and other avian species infected with ornithosis virus (Karrer et al, 1950, Hilleman et al, 1951) *The inhibitory antibody lacks the ability to* combine with guinea pig complement, although it has the capacity to combine with

dicator antibody is obtained from naturally infected pigeons or humans, or from immunized animals, such as the rabbit If, in an avian serum-ornithosis antigen mixture, the inhibiting antibody unites with the antigen no antigen is available to combine with the

fixes the complement in the presence of specific antigen, no free complement is available, and hence no hemolysis occurs when the hemolytic system is added

The conglutinating complement absorption test (CCAT) represents another modification *of the complement-fixation technic* and has been employed in studies of Q fever, vaccinia, influenza and the psittacosis-lymphogranuloma venereum group of infections (Wolfe and Kornfeld, 1948, Stoker et al, 1950, and Hilleman et al, 1951). In this modification, the occurrence of specific antigen-antibody *union is revealed by agglutination* of erythrocytes in the indicator system and not by their lysis. The test system consists of dilutions of the serum *under examination, antigen* and nonhemolytic complement (cat or horse serum) After appropriate incubation of the mixture, the conglutinating system is added, *thus consists of sheep erythrocytes that have* been sensitized with the natural hemagglutinin present in bovine serum and of conglutinin a hemagglutinin *present* in fresh bovine serum which *reacts* with a sensitized cell in the presence of nonhemolytic complement If the test serum contains antibody, complement is

bound, and none is available to react in the conglutinating reaction, so that no agglutination of the erythrocytes occurs The CCAT is not as simple to perform as a direct or hemolytic complement-fixation test, and the advantage of increased sensitivity is offset by this factor, plus the generally higher incidence of anticomplementary and nonspecific reactions

AGGLUTINATION AND PRECIPITATION

In the earlier literature on virology, the in vitro aggregation of viral or rickettsial materials by specific antisera was generally referred to as "flocculation" This broad term, which embodies both agglutination and precipitation, is still used to describe those situations in which it is difficult to determine whether the reacting antigen is a suspension of particles or a solution

When aggregation is due to the clumping of relatively large particles such as elementary bodies or rickettsiae, particles readily seen by ordinary microscopy, the term *agglutination* is permissible and appropriate. The term *precipitation* is reserved for those reactions in which the aggregating material comes out of solution as, for example, the soluble LS antigen of vaccinia However, absolute differentiation of the type of reaction on the basis of the particle size of the reacting antigen is not always feasible—the soluble antigen of *R prowazeki*, for example, is particulate, its size being considerably smaller than that of the rickettsial body itself and approximating that of the smallest viruses

In general, the term "flocculation" should be applied to aggregation effected by specific immune serum in materials known to contain both large particles and soluble antigen, or manifested in materials in which the physical nature of the antigen is uncertain. This is well exemplified by vaccinia virus. Convalescent sera to vaccinia virus contain at least 4 different antibodies, namely, L, S, NP and X (Chap 32), all of which have the capacity to agglutinate the elementary bodies of vaccinia In addition, the L, S and NP antibodies [illegible]

ployed Consequently, aggregates appearing in a mixture of vaccinial antigen and specific antiserum may consist of viral particles agglutinated by any one or all of the antibodies mentioned above, as well as of LS antigen precipitated by either of its antibodies.

Flocculation reactions have been described for a number of viruses including, recently, the viruses of mumps, Newcastle disease, influenza (Belyavin, 1957) and poliomyelitis (Smith et al, 1956). In some cases the concentration of virus present in crude suspensions of infected tissue is adequate to produce visible floccules, but not infrequently such preparations contain inhibitory substances (Belyavin, 1957) The inhibitors may be removed by such simple procedures as adsorption and elution of the virus from erythrocytes (influenza viruses), adsorption and elution followed by further purification by high-speed centrifugation (Newcastle disease virus), or purification by high-speed centrifugation alone (mumps virus). Those viruses which show flocculation in the presence of specific antisera almost invariably possess the capacity to fix complement specifically This is true, for example, of the viruses just mentioned. However, the converse is not always true—a number of viruses which are reactive in the complement-fixation system do not give demonstrable flocculation However, this may be due to differences in the amount of serologically reactive material required to bring about these reactions In general, detectable fixation of complement requires appreciably smaller amounts of antigen than does visible flocculation Infected fluid from HeLa cell cultures of poliomyelitis virus, for example, may be *diluted* 6- to 8-fold or more for use in the complement-fixation test (Schmidt et al, 1957) whereas it must be *concentrated* 40-fold or more to provide a suitable antigen for the flocculation reaction (Smith et al, 1956). Similarly, about 10 times more of a washed suspension of *Rickettsia prowazeki* is required for demonstrable agglutination than is necessary to give specific fixation of complement.

Agglutination tests are feasible with the rickettsiae and with some of the larger mammalian viruses. Several technics, viz, the macroscopic, the microscopic and the capillary, are employed. The macroscopic, or tube, method requires comparatively large amounts

of antigen, and to circumvent this undesirable feature, microscopic and capillary technics have been devised Thus, the macroscopic method can be used for the routine diagnosis of Q fever (Lennette et al 1952), but to conserve materials Babudieri (1953) devised a microscopic technic in which very minute amounts of antigen and antiserum are used, the reagents are mixed in droplet amounts on glass slides, and the reaction is read microscopically. Recently, a capillary tube agglutination test has been described for the detection and the assay of antibody against *Coxiella burnetii* (Luoto, 1956) The antigen which consists of stained rickettsiae is drawn into a capillary tube which is then filled with the serum or other material to be tested for antibody content The ends of the tube are sealed with clay or wax and the tubes are incubated in a vertical position for several hours, the aggregates of agglutinated rickettsiae which form are readily visible macroscopically

As is mentioned below (see Indirect Hemagglutination), red blood cells can be coated with viral antigens, and since they then become agglutinable by specific immune serum such treated cells can be used for antibody assay and even for diagnosis Attempts have been made to utilize other insoluble particles, e g , bacteria (Roberts and Jones, 1941), collodion (Goodner, 1941) or insoluble dyes (Smorodintsev and Fradkina, 1944) as carriers of specific serologically reactive antigens so that in the presence of antibody large, readily visible aggregates would form None of these methods has proved to be entirely satisfactory for diagnostic purposes

More recently Segre (1957) has adapted this fundamental technic to the detection of viral antigens Antibodies against the viruses of hog cholera and of vesicular stomatitis were adsorbed onto finely divided particles of a basic anion exchange resin When such antibody coated resin particles were mixed with serum taken from swine during the viremic phases of infection with these viruses, specific agglutination of the coated particles occurred Serum antibodies to these two viruses can be detected by means of agglutination-inhibition test based on the use of antibody coated resin particles (Segre, 1957).

VIRAL AND RICKETTSIAL HEMAGGLUTINATION

In 1941 Hirst, and McClelland and Hare, independently reported that influenza virus possesses the capacity to agglutinate chicken erythrocytes Subsequently, a miscellany of other viruses has been found to possess hemagglutinative activity The heterogeneity of viruses comprising the group with hemagglutinative properties is indicated by the fact that they range in size from the smallest (e.g , foot and mouth disease virus) to the largest viruses (e g , vaccine virus), possess varying tissue tropisms and vary in the spectrum of red cells which they will agglutinate

Because of differences in the nature of the hemagglutinins produced by different groups of viruses, differences in their behavior, and in the physical and chemical conditions under which they can be demonstrated, etc., it is not possible to treat these agents as a single group characterized by hemagglutinative properties Certain generalizations may be made but it is necessary to discuss the hemagglutinative viruses in terms of groups which exhibit certain features in common

Broadly speaking, agglutination of red cells by viral agents is classifiable into two types of reactions, viz direct hemagglutination and indirect hemagglutination Direct hemagglutination, the type originally described by Hirst and by McClelland and Hare, and sometimes referred to as the Hirst phenomenon, results from the action of the viral particle itself, or of some substance intimately associated with viral synthesis or with the action of the virus on the infected cell, directly upon the erythrocyte Indirect hemagglutination results from exposure of red cells coated with a viral or rickettsial antigen to the action of specific immune serum, the interaction of viral antigen with specific immune serum results in clumping and sedimentation of the sensitized cells

In the case of those viral agents which produce direct hemagglutination, this property can be utilized for assay of the viral content of a preparation and also for quantitation of specific antibody the so-called [illegible]

all those viruses which produce direct hemagglutination, the facility with which quantitation of hemagglutinative capacity or of specific inhibiting antibody can be accomplished varies considerably, ranging from the quite simple test to the relatively complicated procedure

Methods based on red cell agglutination and on specific inhibition of agglutination are, with the exception of the technics dealing with arbor viruses (Chap 12), described in considerable detail elsewhere (American Public Health Association Committee, 1956), the reader is referred to that source For details of the methodology involved in hemagglutination and hemagglutination-inhibition tests with arbor viruses, reference should be made to the original publications (see below).

Direct Hemagglutination

The group of viruses characterized by direct hemagglutinative action may be divided into several subgroups:

The first subdivision includes the viruses of influenza, mumps and Newcastle disease, i e , myxoviruses Because hemagglutination by these viruses occurs under very simple conditions and so is quite readily demonstrable, this

ing description is based Hemagglutination is associated with attachment of the viral particles themselves to the red cell This is not a matter of simple adsorption, since spontaneous elution of virus also occurs and, under proper condition, may even be complete The eluted virus does not appear to be altered in any way, although the red cells are no longer able to adsorb fresh virus of the same type The surface of the red cell contains receptors which are mucoprotein in nature and provide a point for virus attachment. On the surface of the virus elementary body, in turn, are certain chemical groupings or effectors with a pattern complementing that of the receptors on the cell Union between elementary body and red cell is thus a linkage of effector with specific receptor. The virus possesses an enzyme, now identified as a glycosidase (Gottschalk, 1956), with the ability to destroy receptors. Destruction of the receptors leads to release of virus from the surface of the red cell.

The second subdivision is comprised of viruses (variola, vaccinia, ectromelia, meningopneumonitis) whose marked hemagglutinating activity is also readily demonstrable but which, unlike that of the first group, is due to a soluble fraction completely separable from the viral particle The hemagglutinin can be separated from the virus by high-speed centrifugation or by filtration through virus-retaining filters The fact that only the largest viruses have been found, thus far, to have hemagglutinins separable from the viral particle may be significant. As in the case of the first group, the hemagglutinative activity can be inhibited by specific antibody.

The third subdivision consists of the arthropod-borne (arbor) viruses. Uncertainty exists as to whether hemagglutination by these agents is a function of the viral particle itself or of some other factor closely associated with it. Methods for the demonstration of hemagglutinins and specific inhibitory antibodies are based on the findings of a number of workers (Warren et al , 1949; Chanock and Sabin, 1953, Casals and Brown, 1954; Porterfield, 1954; Clarke and Casals, 1955, Clarke and Theiler, 1955) and Rockefeller Foundation Virus Laboratory Technical Manual on Hemagglutination (unpublished, 1955). These methods have been gradually improved, simplified and extended to additional viruses in this group and are now being used as standard procedures in specialized laboratories Although with some arbor viruses hemagglutinins are not as easily demonstrable as the hemagglutinins from the other two subdivisions, it is not, in general, difficult to prepare agglutinating antigens with most members of the arthropod-borne group The usual source of hemagglutinin, infected mouse brain or, in certain instances, infected mouse serum, contain inhibitory substances which may inhibit or mask the reaction and must be removed In addition, the pH range (6 0 to 7 2) at which the antigens are active is narrower than with the other two subdivisions.

Inhibition of hemagglutination is not always due to specific antibody but under some circumstances may be due to nonspecific factors As concerns the myxoviruses, nonspecific inhibitors to these agents are found in a wide variety of biologic substances, e g , fluid from ovarian cysts, egg white, salivary glands, human urine, serum, etc , and appear to be mucoproteins Since the concentration of inhibitors in serum may be so high as to mask

inhibition due to specific antibody, and since its mere presence frequently is sufficient to give rise to misleading diagnostic results, destruction of the inhibitors not infrequently becomes a requisite in diagnostic work. The inhibitors to the myxoviruses can be destroyed by means of receptor destroying enzyme (RDE) from *V. cholerae* filtrates, or by trypsin or potassium periodate.

The inhibitors of the arbor viruses appear to be lipoproteins and can be removed from the serum by extraction with acetone or chloroform, or by adsorption to substances such as kaolin. Development of inhibitory antibodies during the course of infection can be demonstrated with these agents; however, the presence of group cross-reactions often renders the interpretation of the results difficult concerning the specific agent or agents involved in the infection. Where the HA and HI tests have been most helpful is as a screening procedure for sera and as a means for identification and classification of these viruses. By means of this test, the arbor viruses can be classified into at least 3 immunologic groups (Chap. 12). Group A, for example, contains eastern, western and Venezuelan equine encephalomyelitis viruses, Semliki Forest virus, Sindbis, Mayaro, etc., whereas Group B contains Japanese B, St. Louis, Murray Valley encephalitis viruses, Russian spring-summer encephalitis virus, yellow fever virus, dengue virus, etc. In Group C, there are certain viruses (Chap. 19) about which relatively little is known. With a number of the arbor viruses, it has not been possible as yet to demonstrate the existence of hemagglutinins, but there is a suggestion that additional immunologic groups may exist. It is to be recalled that the demonstration of specific or group inhibiting antibodies requires the removal of nonspecific inhibitors from the sera under test. This type of nonspecific inhibitor has been found in the sera from all animal species so far investigated. In this respect, arbor viruses do not differ greatly from certain myxoviruses (see above).

For detailed information regarding the technics that are currently used for the identification and the classification of the arbor viruses the reader is referred to a forthcoming publication by Clarke and Casals (1958).

Indirect Hemagglutination

Certain viruses which, by the usual technics for the demonstration of direct hemagglutination, appear to possess no hemagglutinative properties can be made to agglutinate erythrocytes by an indirect method. Basically, the method depends upon the adsorption of reactive material (whether elementary bodies or viral antigens is uncertain) onto the surface of an essentially inert carrier particle, in this case the erythrocyte. When the specific complementary reagent, namely, antibody, is added, visible aggregation of the carrier particle occurs. Thus, herpes simplex virus can be adsorbed onto sheep erythrocytes whose surface has been altered by prior treatment with tannic acid. When such virus-coated cells are exposed to specific immune serum, hemagglutination occurs (Scott et al., 1957). The method can be used for the quantitation of antibody in serum, and it should be noted that the action of the immune serum is to cause hemagglutination and not, as is true in the direct type of test, to inhibit hemagglutination. A similar hemagglutination test has been described for the quantitation of adenovirus antibodies (Friedman and Bennett, 1957).

Erythrocyte Sensitizing Substance (ESS)

Chang (1953) and Chang et al. (1953) described the isolation of a serologically active heat-stable antigen from typhus fever rickettsiae and subsequently encountered a similar antigen in rickettsiae of the Rocky Mountain spotted fever group (Chang et al., 1954). The serologically active material, designated the erythrocyte sensitizing substance, or ESS, renders human group O erythrocytes susceptible to agglutination by specific immune sera.

The hemagglutination of ESS-treated erythrocytes, the so-called anti-ESS test, may

over the complement-fixation test in that it is not only simpler to perform but also ESS antibodies appear earlier in the course of infection than do complement-fixing antibodies The advantage of the test over macroscopic rickettsial agglutination methods lies in the very small amounts of antigen it requires The test is also reported (Chang et al, 1953) to be superior to the Weil-Felix test, both in its specificity and in its ability to detect antibodies in the sera of patients with Brill's disease (such sera often contain no demonstrable agglutinins to proteus OX-19) With respect to infections by rickettsiae of the Rocky Mountain spotted fever group, the anti-ESS test possesses similar advantages over the conventional diagnostic methods

ESS does not react in direct complement-fixation or precipitin tests, and antibodies against the ESS of epidemic typhus rickettsiae appear to be distinct from those which

cytes. This suggests that ESS and soluble antigen are closely related and raises the possibility that ESS arises from degradation of soluble antigen

The presence in the tissues of mice infected with *R mooseri* of an antigen apparently related to ESS has been described recently (Downs et al, 1955) This antigen, when added to murine typhus antisera, inhibits their ability to agglutinate ESS-treated cells The antigen is heat-stable, does not fix complement and resembles ESS in a number of ways but, unlike ESS, it does not sensitize erythrocytes to the action of specific immune sera

COLD HEMAGGLUTININS AND STREPTOCOCCUS MG AGGLUTININS

These agglutinins appear in the sera of a considerable proportion of patients with primary atypical pneumonia Their presence is diagnostically significant, although neither has any specific relationship to the disease

Primary atypical pneumonia represents a clinical and roentgenographic syndrome of diverse etiology (Chap. 29). Clinically indistinguishable cases may be produced by bacteria, fungi and especially by viruses and rickettsiae of established identity, e.g, influenza A and B, ornithosis and lymphocytic choriomeningitis viruses, *Coxiella burnetii*, etc However, this heterogeneous group of agents is responsible for only a small proportion of the cases clinically and roentgenographically regarded as primary atypical pneumonia. *In addition, patients infected with these agents develop specific antibodies to them but do not develop cold agglutinins or streptococcus MG agglutinins* When the pneumonitides due to these and similar causes are removed from consideration, there remains a quite appreciable residuum whose etiology has been ascribed to several agents, all presumably viral and different from each other (Chap 29).

As a consequence of recent work, primary atypical pneumonia can be divided into 3 groups. The first group consists of cases in which an adenovirus, generally type 4 or type 7, is etiologically responsible, these patients do not develop cold or MG agglutinins. The second category is comprised of cases which develop cold or MG agglutinins, but from which no virus has been isolated, or at least is generally accepted as the responsible etiologic agent The third category consists of cases not classifiable into either of the other 2 groups

Cold agglutinins derive their name from the fact that they possess the capacity to agglutinate human group O erythrocytes at icebox temperatures but not at body tempera-

glutinin is indicated by the fact that it is encountered in a variety of diseases, e g, trypanosomiasis, hemolytic anemias, blackwater fever, etc Cold hemagglutinins may also be encountered in a variety of common respiratory diseases, but in low titer; primary atypical pneumonia appears to be the only respiratory disease in which high titers of cold hemagglutinins are often present The hemagglutinin is generally detectable in the serum during the second week of the disease and reaches maximal titers during the third

or fourth week, gradually declines thereafter and finally disappears As with other viral serologic tests, a 4-fold or greater rise in titer [illegible]

hemagglutinins in a patient's serum should not be regarded as pathognomonic of primary atypical pneumonia

The frequency with which cold hemagglutinins are demonstrable in primary atypical pneumonia averages about 50 per cent (Horsfall, 1947, Finland and Barnes, 1951), the reported frequencies ranging from as low as 30 per cent to as high as 90 per cent Since the titer is generally proportional to the severity of the disease, the disparity in the frequency with which the hemagglutinins have been encountered by various observers may result from differences in severity of the illnesses studied

Streptococcus MG is a nonhemolytic streptococcus which was isolated from the lungs of fatal cases of primary atypical pneumonia by Thomas et al (1945) There is no evidence to incriminate streptococcus MG as the causal agent of primary atypical pneumonia, but it is of interest that during recovery from this disease, many patients develop agglutinins against this organism This streptococcus has been encountered in the respiratory tract of normal individuals It is found not infrequently in patients with acute respiratory disease without a pneumonic component, but it is found much more commonly in cases of primary atypical pneumonia than in other types of acute respiratory disease Similarly, although agglutinins to this streptococcus may be found in conditions other than primary atypical pneumonia, rises in titer have been observed almost entirely in primary atypical pneumonia The agglutinins appear about the second or third week after onset of illness and attain maximum titers during the fourth or fifth week A 4-fold or greater rise in titer is of diagnostic significance, although the presence of a high titer, i e, 1 32 or greater, in a single convalescent phase serum specimen affords presumptive positive evidence of primary atypical pneumonia As is true with cold hemagglutinins, the titer of MG agglutinins attained is correlated with the severity of the disease While many patients develop both cold hemagglutinins and MG agglutinins, an appreciable number develop one but not the other Consequently, it is desirable that sera be tested for the presence of both types of agglutinin

RICKETTSIAL AND VIRAL TOXINS

Clinical descriptions of patients with certain viral and rickettsial diseases, and especially the latter, frequently include remarks upon the toxic appearance of the individual. Experimentally, this clinical impression finds justification in the clear demonstration within recent years that toxic substances are elaborated by, or form an integral part of, certain rickettsiae and viruses. These toxic substances, or toxins as they are generally referred to, are demonstrable only in preparations with very high concentrations of infectious organisms Up to the present time, it has not been possible to separate the toxins from the living micro-organisms.

In 1940, Gildemeister and Haagen reported that mice inoculated intraperitoneally with yolk-sac suspensions heavily infected with murine typhus rickettsiae (*Rickettsia mooseri*) died within 4 to 20 hours Others have reported similar results to follow inoculation with epidemic typhus (*R prowazeki*) or scrub typhus (*R orientalis*) rickettsiae (Cox, 1953) The rapidity with which death ensues indicates that a toxin and not infection is responsible This is supported by the observation that animals that survive the acute illness produced by the original heavy inoculum remain well for some days and then develop the usual signs of infection and die

Attempts to separate a toxin from the infectious particle have thus far been unsuccessful, apparently because of the highly labile nature of the toxin The toxin can be preserved by storage of the infectious suspensions on dry ice, but storage at icebox temperatures leads to marked losses in potency over a matter of days The toxin can be destroyed by heating (56° C), by the addition of diethyl ether or by the addition of formalin (0 3 to 0 4%)

Bengtson et al (1945) showed that addition of formalin to suspensions of epidemic typhus rickettsiae destroyed the lethal capacity of the toxin but preserved its immunogenicity, since inoculation of such noninfectious material into man or guinea pigs elicits an antitoxin response (Topping et al ,

1945) Conversion of toxin into a toxoid by formalinization has also been shown with murine typhus rickettsiae. Similarly, several viruses of the psittacosis-lymphogranuloma venereum group have been found to retain their capacity to produce antitoxin after inactivation. Inactivated preparations of influenza A and B viruses also retain their immunogenic capacity to protect mice against the toxic action of fully infectious preparations (Henle and Henle, 1946a, 1946b).

In general, the antitoxins elicited by rickettsial and viral toxins are highly specific. The antitoxins produced against the rickettsiae of

capacity of each antitoxin for the heterologous toxin However, the toxin of the Gilliam strain of scrub typhus rickettsiae is so very highly specific that it is neutralized by its homologous antiserum but not by antisera to heterologous strains of *R. orientalis* (Smadel et al, 1946) An analogous situation exists with respect to the viruses Thus, the toxic factors of the influenza A and influenza B viruses produce antitoxins which have no neutralizing effect on the toxins of other myxoviruses However, certain viruses within

examined 27 viruses of the psittacosis group and found them classifiable into 6 groups on the basis of toxin neutralization tests in mice. Of these 27 strains, only the toxins and the corresponding antitoxins of the Louisiana and the feline pneumonitis strains were specific; the remaining 25 strains were divisible into 4 groups which showed antigenic overlapping

The existence of toxins has been clearly shown for the rickettsiae of epidemic typhus (*R. prowazeki*), murine typhus (*R mooseri*), scrub typhus (*R. orientalis*), and Rocky Mountain spotted fever (*R. rickettsii*) (Bell

disease, mumps and eastern and western equine encephalomyelitis viruses (Cox, 1953).

The toxins produced by the rickettsiae and by the psittacosis-LGV group of viruses resemble the bacterial exotoxins rather than the bacterial endotoxins The toxic factors of *R prowazeki* recently described by Olitzki et al (1946) and considered by them to resemble the endotoxins of gram-negative bacteria appear to be unrelated to the toxic materials mentioned above, and highly lethal for the mouse.

The symptomatology and the histopathologic changes produced by the mouse-lethal toxins associated with different rickettsiae are quite similar. Within 1 to 2 hours after inoculation, the mice develop weakness and labored breathing, succeeded by prostration and convulsive seizures; most animals die within 1½ to 3 hours after inoculation, but death may be delayed for as long as 18 hours, seldom more. The symptoms produced by the viral toxins are similar, but death may occur from within 1 up to 24 hours, and sometimes longer, depending upon the virus

Mice which succumb to rickettsial toxins show congestion of the visceral blood vessels in general, and of the small intestine in particular. In some instances, the gut appears hemorrhagic. Frequently, however, the mucosa of the small bowel is covered with a yellowish fluid, and there is marked dilatation of the blood vessels in the villi (Neva and Snyder, 1952) Mice which die early after inoculation with a viral toxin show a gross

thrombi in the glomerular capillaries. The liver may show some focal necrosis, but usually the lesions consist of foci of damaged cells containing fat droplets In mice which die at the later stages after inoculation, pulmonary hemorrhage or edema is seldom seen, but a pronounced focal necrosis of the liver is generally present (Rake and Jones, 1944, Henle and Henle, 1946b, Fastier, 1952).

Studies on viral and rickettsial toxins have been conducted almost entirely in mice, although in some instances other species may be used in place of the mouse In some cases the toxic effect of an agent has been demonstrable by the effect produced in animal species other than the mouse Neva and Snyder (1952) reported that the white rat reacts to the toxins of typhus rickettsiae in much the same way as does the white mouse; in addition, the lesions as described above for the white mouse were also seen in the rat, in which they were somewhat more marked The importance of species for the demonstra-

tion or the detection of toxic activity is indicated by the fact that the white rat is approximately 5 times more resistant to the toxin of R. *mooseri* than to the toxin of *R. prowazeki* (Neva and Snyder, 1952) and also by the fact that the cotton rat, unlike the white rat, fails to react to the toxin but succumbs to infection (Neva and Snyder, 1952)

Intra-ocular inoculation of the rabbit with type A or type B influenza virus results in corneal opacity without demonstrable multiplication of the virus; the toxic effect can be prevented by neutralization of the toxin with specific antiserum (Evans and Rickard, 1945) Corneal opacity similar to that produced by the toxin of the influenza viruses can also be produced by intra-ocular inoculation of the eastern and western equine encephalomyelitis viruses (Evans and Bolin, 1946) and mumps virus (Bolin et al, 1950) Intravenous inoculation of the rabbit with influenza virus (Wagner et al, 1949) or western equine encephalomyelitis virus (Fastier, 1952) elicits a febrile response and a leukopenia which would appear to be manifestations of viral toxicity

At the present time, little is known about the nature of the toxins and of their role, if any, in the pathogenesis of disease The toxins have not been utilized for viral or rickettsial diagnostic purposes, but they have been applied to the standardization of typhus fever vaccines (Cox, 1953) and have assisted in some instances in the classification of related viruses (Manire and Meyer, 1950)

SOLUBLE ANTIGENS

During the course of certain viral and rickettsial infections, there are elaborated substances which are serologically active and highly specific for the infecting agent Since these reactive materials are noninfectious and are separable from the infectious agent itself, they are referred to as soluble antigens In its broadest sense, the term includes all the serologically active materials—complement-fixing, precipitating, hemagglutinating, etc, antigens—that are readily separable from the infective viral or rickettsial bodies by simple physical technics In a restricted sense, and the one in which the term is usually employed, the designation soluble antigen or S antigen is understood to refer to complement-fixing material separable from the infective particles

Soluble antigens are smaller in size than the rickettsial or viral agents with which they are associated Obviously, the occurrence of such antigens is recognized most readily among the larger and more complex viruses and the rickettsiae In dealing with viruses of progressively smaller size, it becomes increasingly difficult to determine whether the observed reactivity is attributable to a soluble antigen or to the viral particle per se. Such difficulties are exemplified by yellow fever, in which it has not proved to be possible to determine whether the noninfectious, serologically specific substance found in this disease (Hughes, 1933, Lennette and Perlowagora, 1945) is a soluble antigen within the accepted meaning of that term.

The existence of soluble antigens was first shown by Craigie (1932) in connection with vaccinia virus The antigen, demonstrable by its activity in precipitation and complement-fixation tests, could be separated from the elementary bodies by filtration or by high-speed centrifugation The soluble substance was named LS antigen when it was found to

extractable from elementary bodies (Smadel et al., 1942) and, like the LS antigen, reacts in precipitin and complement-fixation tests Animals inoculated with these antigens produce complement-fixing and precipitating antibodies but do not develop neutralizing antibodies nor do they develop resistance to infection with the virus

Since Craigie's original observations, soluble (complement-fixing) antigens have been described in association with a number of viruses and rickettsiae As is brought out in the chapters dealing with specific diseases, soluble antigens have been described for the psittacosis-lymphogranuloma venereum group of viruses and for the viruses of lymphocytic choriomeningitis, influenza A and influenza B, mumps, etc, and for the rickettsiae of epidemic and murine typhus, Rocky Mountain spotted fever and boutonneuse fever, rickettsialpox and scrub typhus

The association of soluble antigens with rickettsiae was not demonstrated until 1942 (Smadel, 1948) It is of interest that the soluble antigen of *R. prowazeki* differs from

that associated with vaccinia virus, for example, in that the former possesses many of the immunogenic properties of the intact rickettsial body, and consequently it is desirable to include it in vaccines. (A soluble *immunizing* antigen obtained by some disruption of the rickettsiae has been described for Q fever [Colter et al, 1956] No information is available on its serologic reactivity)

The soluble antigens of *R prowazeki* and *R mooseri* are virtually indistinguishable, hence, when used in diagnostic complement-fixation tests, they do not differentiate between infections due to these organisms The cross-reactive materials or antigens can be removed by washing the rickettsial suspensions, for a time, the washed organisms will give essentially type-specific fixation. Disruption of *R prowazeki*, for example, releases a nucleoprotein which reacts as a comparatively type-specific antigen in the complement-fixation test In the presence of proteolytic enzymes, however, type-specificity is destroyed, and the antigen acquires group-specific reactivity (Chambers et al, 1950)

In a similar way, the rickettsia of Rocky Mountain spotted fever shares a soluble antigen in common with boutonneuse fever and with rickettsialpox

Information concerning the nature and the properties of soluble antigens is still relatively meager, and perhaps the most comprehensive body of information available on soluble antigens at the present time is that in connection with influenza viruses

The soluble or S antigen of influenza virus is present in high concentrations in infected tissues Infected cells which release virus release comparatively little S. The antigen is separable from the viral particle by high-speed centrifugation or by adsorption of the elementary bodies onto red cells, since it has a smaller particle size than the elementary body, it is not readily sedimented Also, unlike the virus, the antigen is not adsorbed to red blood cells The S antigen is characterized by possessing *only complement-fixing activity*, whereas the elementary bodies of the virus possess some complement-fixing activity in addition to other properties such as infectivity, hemagglutinating capacity, toxicity, interfering capacity, etc.

The viral, or V, antigens are serologically distinct from the S antigen (Wiener et al, 1946). The S antigen is type-specific, since S antigens obtained from various strains of influenza A virus are identical but are distinct from those obtained from influenza B virus strains (Lennette and Horsfall, 1941; Kirber and Henle, 1950). The V antigens also show cross-reactivity within the virus type but also possess marked strain-specific components within the mosaic that makes up the antigen. As mentioned earlier, the V antigen, as represented by washed elementary bodies, may also contain some S antigen (Lennette and Horsfall, 1940, Kirber and Henle, 1950).

It has been suggested (Fulton, 1949; Kirber and Henle, 1950) that the S antigen is not an integral part of the viral particle but is a by-product resulting from the pathologic action of the virus on the cell Other data, and especially more recent findings, indicate that the S antigen is an integral part of the influenza virus particle (Hoyle, 1952) Hoyle (1952) reported that when the elementary bodies of influenza virus are treated with ether, they disintegrate with the liberation of two types of smaller particles, namely, soluble antigen and hemagglutinin. The experimental work of Lief and Henle (1956a, 1956b) also indicates that the S antigen forms an integral part of the elementary body; they found that washed viral suspensions free of detectable S yielded high titers of S after exposure to ether, which suggests that the viral particle contains large concentrations of S Also pointing to the internal location of the S antigen is the finding that standard virus preparations yield uniform amounts of antigen on ether treatment (Lief and Henle, 1956a), incomplete virus releases less antigen than does complete virus, the amount of S liberated decreasing with increasing incompleteness of the viral moiety (Lief and Henle, 1956b), and that the release of S antigen is associated with nearly complete destruction of infectivity

Recent evidence indicates that the S antigen of influenza virus, and also of fowl plague virus (Schaffer and Zillig, 1954), is a ribose nucleoprotein (Hoyle, 1952; Ada and Perry, 1954; Paucker et al, 1956).

REFERENCES

Ada, G L, and Perry, B T, 1954, Studies on the soluble complement-fixing antigens of influenza virus III The nature of the antigens Australian J Exper Biol & M Sc, 32, 177-186

American Public Health Assoc Committee, 1956, Diagnostic Procedures for Virus and Rickettsial Diseases, ed 2, Francis T, Jr (ed), New York, Am Pub Health Assoc

Andrewes, C H, 1928, The action of immune serum on vaccinia and virus III *in vitro* J Path & Bact *31*, 671-698

Babudieri, B, 1953, Laboratory techniques for diagnosis of Q fever, in Advances in the Control of Zoonoses, pp 193-209, Geneva, World Health Organ, Monograph Series, No 19

Bell, E J, and Pickens, E G, 1953, A toxic substance associated with the rickettsias of the spotted fever group, J Immunol *70*, 461-472

Belyavin, G, 1957, The influenza virus flocculation reaction as a method of antigenic typing, J Hyg *55*, 281-289

Bengtson, I A, Topping, N H, and Henderson, R G, 1945, Epidemic typhus Demonstration of a substance lethal for mice in the yolk sac of eggs infected with *Rickettsia prowazeki* Nat Inst Health Bull (No 183), pp 25-29

Bolin, V S, Anderson, J A, and Leymaster, G R, 1950, Experimental intraocular infection of guinea pigs with mumps virus, Proc Soc Exper Biol & Med *75*, 166-170

Casals, J, 1949, Acetone-ether extracted antigens for complement fixation with certain neurotropic viruses, Proc Soc Exper Biol & Med *70*, 339-343

Casals, J, and Brown, L V, 1954, Hemagglutination with arthropod-borne viruses, J Exper Med *99*, 429-449

Casals, J, and Palacios, R, 1941, The complement fixation test in the diagnosis of virus infections of the central nervous system, J Exper Med *74*, 409-426

Chambers, L A, Cohen, S S, and Clawson, J R, 1950, Studies on commercial typhus vaccines II The antigenic fractions of disrupted epidemic typhus rickettsiae J Immunol *65*, 459-461

Chang, R S M, Murray, E S, and Snyder, J C, 1954, Erythrocyte-sensitizing substances from rickettsiae of the Rocky Mountain Spotted Fever group J Immunol *73*, 8-15

Chang, S M, 1953, A serologically-active erythrocyte sensitizing substance from typhus rickettsiae I Isolation and titration J Immunol *70*, 212-214

Chang, R M, Snyder, J C, and Murray, E S, 1953, A serologically-reactive erythrocyte sensitizing substance from typhus rickettsiae II Serological properties J Immunol *70*, 215-221

Chanock, R M, and Sabin, A B, 1953, The hemagglutinin of St Louis encephalitis virus I Recovery of stable hemagglutinin from the brains of infected mice II Physico-chemical properties and nature of its reaction with erythrocytes III Properties of normal inhibitors and specific antibody, use of hemagglutination-inhibition for diagnosis of infection J Immunol *70*, 271-285, 286-301, 302-316

Clarke, D H, and Casals, J, 1955, Improved methods for hemagglutination with arthropod-borne viruses, Proc Soc Exper Biol & Med *88*, 96-99

———, 1958, Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses, Am J Trop Med *7*, 561-573

Clarke, D H, and Theiler, M, 1955, The hemagglutinins of Semliki Forest and Bunyamwera viruses, their demonstration and use, J Immunol *75*, 470-474

Colter, J S, Brown, R A, Bird, H H, and Cox, H R, 1956, The preparation of a soluble immunizing antigen from Q-fever rickettsiae, J Immunol *76*, 270-274

Cox, H R, 1953, Viral and Rickettsial toxins, Ann Rev Microbiol *7*, 197-218

Craigie, J, 1932, The nature of the vaccinia flocculation reaction, and observations on the elementary bodies of vaccinia, Brit J Exper Path *13*, 259-268

———, 1939, Die Virusarten als Antigene und die erworbene Immunität gegen Virusinfektionen I Die Antigenfunktionen und die serologischen Reaktionen der Virusarten *in vitro*, in Doerr, R, und Hallauer, C, Handbuch der Virusforschung, Zweite Hälfte, Wien, Springer, 1106-1147

Craigie, J, and Tulloch, W J, 1931, Further investigations on the variola-vaccinia flocculation reaction Special Report Series, No 156, Med Res Council, London, His Majesty's Stat Off

DeBoer, C J, and Cox, H R, 1947, Specific complement-fixing diagnostic antigens for neurotropic virus diseases, J Immunol *55*, 193-204

Downs, C M, Fevurly, J, and Meyer, M M, 1955, Studies on hemagglutination-inhibition phenomena I The presence of inhibiting antigen in typhus-infected animals J Immunol *75*, 35-42

Dulbecco, R, and Vogt, M, 1954a, Plaque formation and isolation of pure lines with poliomyelitis viruses, J Exper Med *99*, 167-182

———, 1954b, One-step growth curve of Western equine encephalomyelitis virus on chicken embryo cells grown *in vitro* and analysis of virus yields from single cells, J Exper Med *99*, 183-199

Dulbecco, R, Vogt, M, and Strickland, A G R, 1956, A study of the basic aspects of neutralization of two animal viruses, Western equine encephalitis virus and poliomyelitis virus, Virology *2*, 162-205

España, C, and Hammon, W McD, 1948, An improved benzene-extracted complement-fixing antigen applied to the diagnosis of the arthropod-borne virus encephalitides, J Immunol *59*, 31-44

Evans, C A, and Bolin, V S, 1946, Corneal reaction to viruses of equine encephalomyelitis after intra-ocular injection, Proc Soc Exper Biol & Med *61*, 106-109

Evans, C A, and Rickard, E R, 1945, The toxic effect of influenza virus in the rabbit eye, Proc Soc Exper Biol & Med *58*, 73-74

Fastier, L B, 1952, Toxic manifestations in rabbits and mice associated with the virus of Western equine encephalomyelitis, J Immunol *68*, 531-541

Finland, M, and Barnes, M W, 1951, Cold agglutinins VII Tests for cold isohemagglutinins in pneumonia and other acute respiratory infections over a four year period Am J M Sc *221*, 152-157

French, E L, 1952, Murray Valley encephalitis isolation and characterization of the aetiological agent, M J Australia *1*, 100-103

Friedman, M, and Bennett, C R, 1957, A hemagglutination test for detection of adenovirus antibodies, Proc Soc Exper Biol & Med *94*, 712-717

Fulton, F, 1949, Growth cycle of influenza virus, Nature, London *164*, 189-190

Gildemeister, E, and Haagen, E, 1940, Fleckfieberstudien I Mitteilung Nachweis eines Toxins in Rickettsien-Eikulturen (Rickettsia mooseri), Deutsche med Wchnschr *66*, 878-880

Ginsberg, H S, and Horsfall, F L, Jr, 1949, A labile component of normal serum which combines with various viruses Neutralization of infectivity and inhibition of hemagglutination by the component J Exper Med *90*, 475-495

Goodner, K, 1941, Collodion fixation a new immunological reaction, Science *94*, 241-242

Gottschalk, A, 1956, The linkage of sialic acid in mucoprotein, Biochim et biophys acta *20*, 560-561

Havens, W P, Jr, Watson, D W, Green, R H, Lavin, G I, and Smadel, J E, 1943, Complement fixation with the neurotropic viruses J Exper Med *77*, 139-153

Henle, G, and Henle, W, 1946a, Studies on the toxicity of influenza viruses I The effect of intracerebral injection of influenza viruses J Exper Med *84*, 623-637

Henle, W, and Henle, G, 1946b, Studies on the toxicity of influenza viruses II The effect of intraabdominal and intravenous injection of influenza viruses J Exper Med *84*, 639-660

Hilleman, M R, Haig, D A, and Helmold, R J, 1951, The indirect complement fixation, hemagglutination and conglutinating complement absorption tests for viruses of the psittacosis lymphogranuloma venereum group J Immunol *66*, 115-130

Hilleman, M R, and Werner, J H, 1954, Recovery of new agent from patients with acute respiratory illness, Proc Soc Exper Biol & Med *85*, 183-188

Hirst, G K, 1941, The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus, Science *94*, 22 23

Horsfall, F L., Jr, 1947, Primary atypical pneumonia, Ann Int Med *27*, 275-281

Howitt, B F., 1937, The complement fixation reaction in experimental equine encephalomyelitis, lymphocytic chorio-meningitis and the St Louis type of encephalitis, J Immunol *33*, 235-250

Hoyle, L, 1952, Structure of the influenza virus The relation between biological activity and chemical structure of virus fractions J Hyg *50*, 229-245

Huebner, R J, Rowe, W P, Ward, T G., Parrott, R H, and Bell, J A, 1954, Adenoidal-Pharyngeal-Conjunctival agents A newly recognized group of common viruses of the respiratory system New Eng J Med *251*, 1077-1086

Hughes, T P, 1933, A precipitin reaction in yellow fever, J. Immunol *25*, 275-294

Hummeler, K, Kravis, L. P, and Sigel, M M, 1952, A rapid method for the identification of newly isolated influenza viruses, J Bact *64*, 253-257

Jawetz, E, and Coleman, V R, 1952, Studies on herpes simplex virus III The neutralization of egg-adapted herpes virus by human sera *in ovo* J Immunol *68*, 645-653

Karber, G, 1931, Beitrag zur kollecktiven Behandlung pharmakologischer Reihenversuche Arch exper Path u Pharmakol *162*, 480-483

Karrer, H, Meyer, K F, and Eddie, B, 1950, The complement fixation inhibition test and its application to the diagnosis of ornithosis in chickens and in ducks I Principles and technique of the test J Infect Dis *87*, 13-23

Karzon, D T, 1956, Non-specific viral inactivating substance (VIS) in human and mammalian sera Natural antagonists to the inactivator of Newcastle disease virus and observations on the nature of the union between the inactivator and virus J Immunol *76*, 454-463

Kidd, J G, and Friedewald, W F, 1942, A natural antibody that reacts *in vitro* with a sedimentable constituent of normal tissue cells I Demonstration of the phenomenon J. Exper Med *76*, 543-556

Kirber, M W, and Henle, W, 1950, A comparison of influenza complement fixation antigens derived from allantoic fluids and membranes, J Immunol *65*, 229-244

LeBouvier, G L, Laurence, G D, Parfitt, [illegible] M, Jennens, M G, and Goffe, A P, 1954, Typing of poliomyelitis virus by complement fixation, Lancet *2*, 531-532

Lennette, E H, 1956, General principles underlying laboratory diagnosis of viral and rickettsial infections, *in* Diagnostic Procedures for Virus and Rickettsial Diseases, Francis, T, Jr (ed), pp 1-52, New York, Am Pub Health Assoc

Lennette, E H, Clark, W H, Jensen, F W, and Toomb, C J, 1952, Q fever studies XV Development and persistence in man of complement-fixing and agglutinating antibodies to *Coxiella burnetii* J Immunol *68*, 591-598

Lennette, E H, and Horsfall, F L, Jr, 1940, Studies on epidemic influenza virus The nature and properties of the complement-fixing antigen J. Exper Med *72*, 233-246

———, 1941, Studies on influenza virus The complement-fixing antigen of influenza A and swine influenza viruses J Exper. Med *73*, 581-599

Lennette, E H, and Perlowagora, A, 1943, The complement fixation test in the diagnosis of yellow fever Comparative value of the serologic and histopathologic methods of diagnosis Am J Trop Med *23*, 11-18

Lennette, E H, Wiener, A, Neff, B J, and Hoffman, M N, 1956a, A chick embryo-derived complement-fixing antigen for Western equine encephalomyelitis, Proc Soc Exper Biol & Med *92*, 575-577

Lennette, E H, Wiener, A, Ota, M I, Fujimoto, F. Y, and Hoffman, M N, 1956b, Rapid identification of isolates of Western equine encephalomyelitis virus by the complement fixation technique, Am J Hyg *64*, 270-275

Lief, F S, and Henle, W., 1956a, Studies on the soluble antigen of influenza virus I The release of S antigen from elementary bodies by treatment with ether Virology 2, 253-271

———, 1956b, Studies on the soluble antigen of influenza virus III The decreased incorporation of S antigen into elementary bodies of increasing incompleteness Virology 2, 782-797

Lipton, M M., and Steigman, A J, 1955, A simplified colorimetric test for poliomyelitis virus and antibody, Proc Soc Exper Biol & Med 88, 114-118

Luoto, L, 1956, A capillary-tube test for antibody against *Coxiella burnetii* in human, guinea pig and sheep sera, J Immunol 77, 294-298

McClelland, L, and Hare, R, 1941, The adsorption of influenza virus by red cells and a new *in vitro* method of measuring antibodies for influenza virus, Canad Pub Health J 32, 530-538

Maltaner, F., 1946, Significance of thromboplastic activity of antigens used in complement fixation tests, Proc Soc Exper Biol & Med 62, 302-304

Manire, G P and Meyer, K F 1950, The toxins of the psittacosis-lymphogranuloma venereum group of agents III Differentiation of strains by the toxin neutralization test J Infect Dis 86, 241-250

Neva, F A, and Snyder, J C, 1952, Studies on the toxicity of typhus rickettsiae, J Infect Dis 91, 72-78

Olitzki, L, Czaczkes, J W, and Kuzenok, A 1946 Endotoxic factors of *Rickettsia prowazeki* and their immunological relationship to the endotoxins of other gram-negative microorganisms, J Immunol 53, 365-370

Paucker, K, Lief, F S, and Henle, W, 1956, Studies on the soluble antigen of influenza virus IV Fractionation of elementary bodies labeled with radioactive phosphorus Virology 2, 798-810

Pereira, H G, 1956, Typing of Adenoidal-Pharyngeal-Conjunctival (APC) viruses by complement fixation, J Path & Bact 72, 105 109

Porterfield, J S, 1954, The haemagglutination inhibition test in the diagnosis of yellow fever in man, Tr Roy Soc Trop Med & Hyg 48, 261 266

Rake, G, and Jones, H P, 1944, Studies on lymphogranuloma venereum II The association of specific toxins with agents of the lymphogranuloma-psittacosis group, J Exper Med 79, 463-486

Reed, L J, and Muench H, 1938, A simple method of estimating fifty per cent endpoints, Am J Hyg 27, 493-497

Rice, C E, 1948, Inhibitory effects of certain avian and mammalian antisera in specific complement fixation systems, J Immunol 59, 365-378

Roberts, [redacted] C, and Jones, L R, 1941, Agglutination of encephalitis virus coated bacterial cells by virus antisera, Proc Soc Exper Biol & Med 47, 75-76

Robertson, H E, Brunner, K T, and Syverton, J T, 1955, Propagation *in vitro* of poliomyelitis viruses VII pH change of HeLa cell cultures for assay Proc. Soc Exper Biol & Med 88, 119-122

Rockefeller Foundation Virus Laboratory, 1955, Technical Manual on Hemagglutination (unpublished)

Sabin, A. B, 1951, Hemagglutination by viruses affecting the human nervous system, Fed Proc 10, 573-578

Sabin, A B, and Buescher, [redacted] L, 1950, Unique physico-chemical properties of Japanese B encephalitis virus hemagglutinin, Proc Soc Exper Biol & Med 74, 222-230

Salk, J E, Youngner, J S, and Ward, E N, 1954, Use of color change of phenol red as the indicator in titrating poliomyelitis virus or its antibody in a tissue culture system, Am J Hyg 60, 214-230

Schäfer, W, and Zillig, W, 1954, Über den Aufbau des Virus-Elementarteilchens der klassischen Geflügelpest I Gewinnung, physikalisch-chemische und biologische Eigenschaften einiger Spaltprodukte, Ztschr Naturforsch 9b, 779-788

Schmidt, N J, and Lennette, [redacted] H, 1955, A complement fixation test for poliomyelitis, J Exper Med 102, 133-150

———, 1957, An inquiry into the use of the complement fixation test for the typing of poliomyelitis viruses, Am J Hyg 66, 119-130

Schmidt, N J, Lennette, E H, Doleman, J H, and Hagens, S J, 1957, Factors influencing the potency of poliomyelitis complement-fixing antigens produced in tissue culture systems, Am J Hyg 66, 1-19

Scott L V, Felton, F G, and Barney, J A, 1957, Hemagglutination with herpes simplex virus, J Immunol 78, 211-213

Segre, D, 1957, A new serologic test for the detection of viral antigens its application to the viruses of hog cholera and vesicular stomatitis, J Immunol 78, 304-309

Smadel, J E, 1948 Complement fixation and agglutination reactions in rickettsial diseases, *in* The Rickettsial Diseases of Man, pp 190-197, Washington, Am Assoc Advancement of Sc

Smadel, J E, Baird, R D, and Wall, M J, 1939, Complement fixation in infections with the virus of lymphocytic choriomeningitis, Proc Soc Exper Biol & Med 40, 71-73

Smadel, J E, Jackson, E B, Bennett, B L, and Rights, F L, 1946, A toxic substance associated with the Gilliam strain of *R orientalis*, Proc Soc Exper Biol [redacted] Med 62, 138-140

Smadel, J E, Rivers, T M, and Hoagland, C L, 1942, Nucleo-protein antigen of vaccine virus I A new antigen from elementary bodies of vaccinia Arch Path 34, 275-285

Smadel, J E, and Wall, M J, 1941, Identification of the virus of lymphocytic choriomeningitis, J Bact 41, 421-430

Smith, W, Sheffield, F W, Churcher, G, and Lee, L H, 1956, Poliomyelitis virus flocculation by a micro-method, Lancet 2, 163-165

Smorodintsev, A A, and Fradkina, R V, 1944, Slide agglutination test for rapid diagnosis of pre-eruptive typhus fever, Proc Soc Exper Biol & Med 56, 93-94

Sosa-Martinez, J, and Lennette, [redacted] H, 1955, Studies on a complement fixation test for herpes simplex, J Bact 70, 205-215

French, E L, 1952, Murray Valley encephalitis isolation and characterization of the aetiological agent, M J Australia *1*, 100-103

Friedman, M, and Bennett, C R, 1957, A hemagglutination test for detection of adenovirus antibodies, Proc Soc Exper Biol & Med *94*, 712-717

Fulton, F, 1949, Growth cycle of influenza virus, Nature, London *164*, 189-190

Gildemeister, E, and Haagen, E, 1940, Fleckfieberstudien I Mitteilung. Nachweis eines Toxins in Rickettsien-Eikulturen (Rickettsia moosen), Deutsche med Wchnschr *66*, 878-880

Ginsberg, H S, and Horsfall, F L, Jr, 1949, A labile component of normal serum which combines with various viruses. Neutralization of infectivity and inhibition of hemagglutination by the component J Exper Med *90*, 475-495

Goodner, K, 1941, Collodion fixation a new immunological reaction, Science *94*, 241-242

Gottschalk, A, 1956, The linkage of sialic acid in mucoprotein, Biochim et biophys acta *20*, 560-561

Havens, W P, Jr, Watson, D W, Green, R H, Lavin, G I, and Smadel, J E, 1943, Complement fixation with the neurotropic viruses J Exper Med *77*, 139-153.

Henle, G, and Henle, W, 1946a, Studies on the toxicity of influenza viruses I The effect of intracerebral injection of influenza viruses J Exper Med *84*, 623-637

Henle, W, and Henle, G, 1946b, Studies on the toxicity of influenza viruses II The effect of intraabdominal and intravenous injection of influenza viruses J Exper Med *84*, 639-660

Hilleman, M R, Haig, D A, and Helmold, R J, 1951, The indirect complement fixation, hemagglutination and conglutinating complement absorption tests for viruses of the psittacosis-lymphogranuloma venereum group J Immunol *66*, 115-130

Hilleman, M R, and Werner, J H, 1954, Recovery of new agent from patients with acute respiratory illness, Proc Soc Exper Biol & Med *85*, 183-188

Hirst, G K, 1941, The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus, Science *94*, 22-23.

Horsfall, F L, Jr, 1947, Primary atypical pneumonia, Ann Int Med *27*, 275-281

Howitt, B F, 1937, The complement fixation reaction in experimental equine encephalomyelitis, lymphocytic chorio-meningitis and the St Louis type of encephalitis, J Immunol *33*, 235-250

Hoyle, L, 1952, Structure of the influenza virus The relation between biological activity and chemical structure of virus fractions J. Hyg *50*, 229-245

Huebner, R J, Rowe, W P, Ward, T G, Parrott, R H, and Bell, J A, 1954, Adenoidal-Pharyngeal-Conjunctival agents A newly recognized group of common viruses of the respiratory system New Eng J Med *251*, 1077-1086

Hughes, T P, 1933, A precipitin reaction in yellow fever, J. Immunol. *25*, 275-294

Hummeler, K, Kravis, L P, and Sigel, M M, 1952, A rapid method for the identification of newly isolated influenza viruses, J Bact *64*, 253-257

Jawetz, E, and Coleman, V R, 1952, Studies on herpes simplex virus III The neutralization of egg-adapted herpes virus by human sera *in ovo* J Immunol *68*, 645-653

Karber, G, 1931, Beitrag zur kollecktiven Behandlung pharmakologischer Reihenversuche Arch exper Path u Pharmakol *162*, 480-483

Karrer, H, Meyer, K F, and Eddie, B, 1950, The complement fixation inhibition test and its application to the diagnosis of ornithosis in chickens and in ducks I Principles and technique of the test J Infect Dis *87*, 13-23.

Karzon, D T, 1956, Non-specific viral inactivating substance (VIS) in human and mammalian sera Natural antagonists to the inactivator of Newcastle disease virus and observations on the nature of the union between the inactivator and virus J Immunol *76*, 454-463.

Kidd, J. G, and Friedewald, W F, 1942, A natural antibody that reacts *in vitro* with a sedimentable constituent of normal tissue cells I Demonstration of the phenomenon J Exper Med *76*, 543-556

Kirber, M W, and Henle, W, 1950, A comparison of influenza complement fixation antigens derived from allantoic fluids and membranes, J Immunol *65*, 229-244

LeBouvier, G L, Laurence, G D, Parfitt, E M, Jennens, M G, and Goffe, A P, 1954, Typing of poliomyelitis virus by complement fixation, Lancet *2*, 531-532

Lennette, E H, 1956, General principles underlying laboratory diagnosis of viral and rickettsial infections, *in* Diagnostic Procedures for Virus and Rickettsial Diseases, Francis, T, Jr (ed), pp 1-52, New York, Am Pub Health Assoc

Lennette, E H, Clark, W H, Jensen, F W, and Toomb, C J, 1952, Q fever studies XV Development and persistence in man of complement-fixing and agglutinating antibodies to *Coxiella burnetii* J Immunol *68*, 591-598

Lennette, E H, and Horsfall, F L, Jr, 1940, Studies on epidemic influenza virus The nature and properties of the complement-fixing antigen J Exper Med *72*, 233-246

———, 1941, Studies on influenza virus The complement-fixing antigen of influenza A and swine influenza viruses J Exper Med *73*, 581-599

Lennette, E H, and Perlowagora, A, 1945, The complement fixation test in the diagnosis of yellow fever Comparative value of the serologic and histopathologic methods of diagnosis Am J Trop Med *25*, 11-18

Lennette, E H, Wiener, A, Neff, B J, and Hoffman, M N, 1956a, A chick embryo-derived complement-fixing antigen for Western equine encephalomyelitis, Proc Soc Exper Biol & Med *92*, 575-577

Lennette, E H, Wiener, A, Ota, M I, Fujimoto, F Y, and Hoffman, M N, 1956b, Rapid identification of isolates of Western equine encephalomyelitis virus by the complement fixation technique, Am J Hyg *64*, 270-275

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11

Principles and Methods of Epidemiology

INTRODUCTION

By derivation, epidemiology would seem to be concerned with the explanation of epidemics of disease in human populations While this definition still obtains in part, naturally with the advance in biologic and medical sciences the field included under epidemiology has broadened considerably (Frost, 1927, Maxcy, 1941, Clark and Leavell, 1953, Taylor and Knowelden, 1957) An epidemic is commonly a sudden increase in the prevalence of a disease which is more or less constantly present or endemic in a community To explain the sudden increase it is necessary to *understand the factors which determine the* usual or interepidemic levels of prevalence and the characteristic distributions which the disease manifests in human populations

The widening horizon of the biologic universe has extended use of the term "epidemiology" to the study of disease in animal populations and plant life Although "enzootic" and "epizootic" have been used by veterinarians to describe the level of prevalence of diseases in animal populations, it is now considered good usage to refer to the epidemiology of cattle plague or of foot and mouth disease While it is etymologically correct to use "epiphytic" *to refer to an outbreak of* infectious disease in plants, a modern plant pathologist may prefer to describe his observations on the stem rust of wheat under the title of epidemiology Such usages are justified by the derivation of the word "epidemic," which, literally translated from the Greek, *means "upon the population"* The population may be composed of people, animals, birds, fish, plants, or whatnot Moreover, while in its early history epidemiology was largely concerned with infectious diseases, it has been found profitable to make a similar approach to the understanding of diseases of unknown etiology, conditions due to nutritional deficiencies, to mental disorders, relationship of excessive smoking to lung cancer, to blood pressure readings, to abnormal cell growth, and even to casualties caused by physical or chemical agents, accidents, etc

Thus, usage has extended the meaning of epidemiology beyond its original limits to designate not merely the doctrine of epidemics but a science of broader scope in relation to the mass-phenomena of diseases in their usual as well as their epidemic occurrences The subject is pursued here with homocentric bias, since our concern is primarily with human welfare For present purposes, therefore, *epidemiology is defined as that field of medical science which is concerned with the relationships of the various factors and conditions which determine the frequencies and the distributions of an infectious process, a disease, or a physiologic state in a human community* It seeks to advance rational conceptual schemes of causation of various ills that afflict mankind, medically speaking To the extent that this body of knowledge is advanced and valid, it becomes possible for appropriate community agencies to take effective meas-

Sternberg, G M, 1892, Practical results of bacteriologic researches, Tr A Am Physicians *7*, 68-86

Stoker, M G P, Coombs, R R A, and Bedson, S P, 1950, The application of the conglutinating complement absorption test to virus systems, Brit J Exper Path *31*, 217-232

Thomas, L, Mirick, G S, Curnen, E C, Ziegler, J E, Jr, and Horsfall, F L, Jr, 1945, Studies on primary atypical pneumonia II Observations concerning the relationship of a non hemolytic streptococcus to the disease J Clin Invest *24*, 227-240

Topping, N H, Henderson, R G, and Bengtson, I A, 1945, Epidemic typhus fever studies of epidemic typhus vaccine Nat Inst Health Bull (No 183), pp 65-86

Topping, N H, and Shepard, C C, 1946, The preparation of antigens from yolk sacs infected with rickettsiae, Pub Health Rep *61*, 701-707

Wagner, R R, Bennett, I L, and LeQuire, V S, 1949, The production of fever by influenzal viruses I Factors influencing the febrile response to single injections of virus J Exper Med *90*, 321-334

Warren, J, Weil, M L, Russ, S B, and Jeffries, H, 1949, Purification of certain viruses by use of protamine sulfate, Proc Soc Exper Biol & Med *72*, 662-664

Wedgwood, R J, Ginsberg, H S, and Pillemer, L, 1956, The properdin system and immunity VI The inactivation of Newcastle disease virus by the properdin system J. Exper. Med *104*, 707-725

Whitney, E, Kraft, L M, Lawson, W B, and Gordon, I, 1953, Noninfectious complement-fixing antigen from embryonated hens' eggs infected with lymphocytic choriomeningitis virus, Proc Soc Am Bact p *50*

Wiener, M, Henle, W, and Henle, G, 1946, Studies on the complement fixation antigens of influenza viruses types A and B, J Exper Med *83*, 259-279

Wolfe, D M, and Kornfeld, L, 1948, Conglutinating complement absorption test compared with hemolytic complement fixation reactions using Q fever immune bovine serum, Proc Soc Exper Biol & Med *69*, 251-255

Wolfe, D M, Kornfeld, L, and Markham, F S, 1949, Simplified indirect complement fixation test *applied to Newcastle disease* immune avian serum, Proc Soc Exper Biol & Med *70*, 490-494.

Wolfe, D M, Vanderscheer, J, Clancy, C F, and Cox, H R, 1946, A method for the preparation of complement-fixing antigens in a study of experimental tsutsugamushi disease (scrub typhus), J Bact *51*, 247-255

Youngner, J S, 1954, Monolayer tissue cultures I Preparation and standardization of suspensions of trypsin-dispersed monkey kidney cells Proc Soc Exper Biol & Med *85*, 202-205

channels to regional lymph nodes, after which it must travel by way of the lymphatics in a stepwise multiplication and invasion, bypassing the defensive barriers in the phagocytic and serologic activity of the host tissues, ultimately to gain admission to the general circulation and select the organs or tissue cells for which it has a predilection and in which it can multiply, as is the case in such diseases as infectious hepatitis. Thus, the incubation period may be a matter of hours, days, weeks, or even months, but for any particular disease its length is relatively constant and predictable. However, as with any other measurable biologic attribute, the incubation period of a specific disease manifests a constant range of variation. Frequency distributions of incubation periods bear a resemblance to the normal curve with a slight skewness, rising more abruptly on the short side of the mean and tailing off on the long side. As pointed out by Sartwell (1950), the degree of variation in relation to the magnitude of the mean has a constant statistical pattern, no matter whether the unit of time be hours, days, weeks or months. The range of the incubation period is an important epidemiologic characteristic for each disease. It determines the time interval necessary for comparison with dates of onset of cases in judging whether an outbreak is due to a common vehicle of dissemination or not, and, when an epidemic is due to transmission from person to person by contact, it determines the rapidity of spread of a disease through a population.

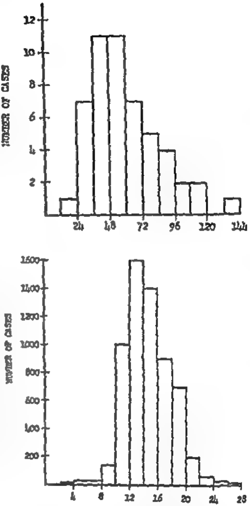

FIG 50 Frequency distributions of incubation periods (*Top*) Epidemic of food-borne streptococcal sore throat (scarlet fever) (in hours). (*Bottom*) A series of cases of serum hepatitis following administration of icterogenic lots of yellow fever vaccine (in weeks) (Sartwell, P. E., 1950, Am J Hyg *51*, 311)

HOST-PARASITE INTERACTION

The second critical stage in the host-parasite relationship mentioned above is the one which may give rise to symptoms and signs of illness by which a disease is recognized. The host reaction varies both in severity and duration. A case of an infectious disease is a host reaction of sufficiently characteristic intensity and duration to permit clinical diagnosis. Reactions which are less intense and of shorter duration are called *abortive* or *suspected* cases, the pattern being too indefinite or protean in nature to permit clinical diagnosis, except in association with *frank* cases. When the subjective and objective symptoms are so slight as to pass unnoticed, the host is said to suffer from an *inapparent* infection. Infections which are below the threshold of clinical recognition are grouped together as *subclinical*. They can be identified by laboratory procedures such as cultural recovery of the infecting micro-organism from the host's tissues, change in the response of the skin to specific antigenic material, or change in a serologic reaction from a negative to positive. It is at least theoretically possible that an

ures directed toward prevention, control or eradication As defined, the field is a broad one.

With the advances in the field of general biology and the collateral medical sciences during the past century, the principal concept of infectious disease gradually became evident It was *comprehensively formulated* by Theobald Smith (1934). ***Infectious disease is a manifestation of parasitism*** Simple though this concept seems today, its formulation marked an important transition The medical explanation of an infectious disease in man broadened to become a biologic one *Finally, it was realized* and accepted that infection should not be set apart as peculiarly within the province of human medicine but was viewed in its natural relationships as an expression of the eternal struggle of living things for food by predation, or parasitism, for shelter and for propagation of their kind. *More particularly* it is the reaction between one of the higher forms of life to the invasion of its tissues by some species of microparasite This conception carries with it implications that are fundamental and far-reaching It affords a framework or pattern into which endless scattered observations can be fitted Explanation of epidemics can then be sought in host-parasite relationships and the environmental factors which modify them For present purposes and ideas, discussion is centered on the principles of epidemiology as applied to understanding and control of viral and rickettsial infections

HOST-PARASITE RELATIONSHIPS

As a result of centuries of host wanderings, mutation and selective adaptation, the viruses and the rickettsiae considered in this book have in some degree become established in the biologic orbit of man and are responsible for some of his ills Their potentialities range from those which only rarely and inadvertently invade his tissues to cause an occasional sporadic case of a rare disease (such as Q fever) to those which are dependent upon human *tissues for their continuous propaga-* ... *to epi-* ...ons and ...ortance of each viral and rickettsial species to man has been determined by a few biologic principles to which only brief reference is made

Man may be an obligate, a principal or an occasional host species, according to the degree of success which a particular species of parasite has in passing through *4 critical stages in relationship to him* A *micro-organism may become completely dependent upon man* for its survival if it is continually successful (1) in finding entrance into his body through its proper ***portal of entry***, whether it be the mucous membrane of the respiratory, the alimentary or the genito-urinary tracts, or the skin by means of trauma or insect bite: (2) *in reaching the particular organ, tissue or* cells in which nutritive conditions are favorable for ***multiplication***. (3) in making an exit from the body in excretions, secretions, or by blood-sucking insects. (4) in surviving under the conditions of the external environment, or in an insect vector, a sufficient time to reach a new susceptible host *To the extent* that it is unsuccessful in maintaining chain passage through these 4 critical stages in human populations and their environment it must be able to utilize other host species or survival mechanisms Thus, one of the first requisites *of a rational explanation of* the behavior of an infectious disease in a human population is to understand to what extent man as a host bears responsibility for the continuous propagation of the causative microparasite (contagious) or shares this responsibility with other species—animal, bird or insect (noncontagious infection).

INCUBATION PERIOD

The first stage in infection is the incubation period, i e , the interval of time from primary invasion through the skin or the mucous membranes to the onset of symptoms of the disease *Its length depends upon the peculiarities of* each particular host-parasite relationship. It is short, a matter of days, when the microparasite has more or less direct access to the tissue in which the primary multiplication takes place, as in certain air-borne infections caused by microparasites which multiply in *the epithelial cells lining the respiratory tract;* influenza is an example *It is longer in those* infections in which the microparasite is unable to multiply at the site of entrance into the body but must make its way through lymph

only with the species of microparasites but frequently with different strains of the same species

SUCCESSFUL PARASITISM

Successful adaptation of a species of microparasite to the human host does not imply a high order of pathogenicity Rather the contrary is true Success for a parasite as for any other living organism, can be measured only by the size of the population of its kind and its ability to survive and maintain these numbers in a constantly changing natural universe There is no advantage if its host sickens and dies, since dissemination of its progeny accordingly becomes limited and soon ceases The opportunities for scatter and chance of productive contact are increased in proportion to the length of time it can continue to multiply and find easy egress in large numbers from a host which is ambulatory and gregarious Accordingly, a high case-fatality rate may be a disadvantage to survival of a parasitic agent Conversely, a low ratio of clinical to subclinical infections and a long duration of the infectious period tend to ensure wide dissemination The microparasites best adapted for survival are those which cause infection with the least inconvenience and injury to the host and create only a low-grade immunity of short duration, a notable exception is rabies

COMMUNITY SUSCEPTIBILITY

A human community is made up of a number of individuals who vary not only in their genetic capacity to react but nearly always in previous experience with the predominant strains of the particular species of microparasite or its close relatives Some individuals have acquired a complete immunity some a partial immunity, some none The proportion of a population at any one time which has little or no immunity determines the theoretical *susceptibility status* or the mass susceptibility of a community for the infectious disease which a specific microparasite causes If a micro-organism is commonly prevalent in a community, the proportion is a constantly changing one as susceptibles are infected, develop immunity and recover If the natural immunity conferred by an infection is durable then susceptibility decreases with age, and the age distribution of cases is consequently that of a *children's* disease If the immunity conferred is temporary, as with many acute respiratory infections, the same individual may be reinfected, and consequently the disease attacks all ages—adults and old people as well as infants and children Thus, community susceptibility has an age distribution, which is specific for each infectious agent, the range of which is indicated by the reciprocal, the age distribution of cases of the disease which it causes provided that exposure is a common experience

With some infections it is possible by means of a skin test or a serologic test for specific antibodies, obtained on a representative sample of persons, to establish the immunity status of a community A common example of the former is the use of the Schick test to determine a community's susceptibility to diphtheria An illustration of the latter is the extensive use of the neutralization test or complement-fixation test as an indication of past infection with yellow fever virus in mapping out the geographic areas in which it is or has been prevalent By ascertaining the correlation of positive sera with age it is possible to discover whether the disease has been prevalent recently or to determine the period of time which has elapsed since the population was last exposed Thus with adequate representation of all ages in the samples of sera tested, if the positive sera were distributed randomly with regard to age and indicated infections in early life, it could be inferred that the exposure was continuing If, on the other hand, the positive sera came only from persons beyond a certain age as for example, beyond the age of 20, it could be inferred that the infection had not been prevalent in the area for the past 20 years

TRANSMISSION

The qualitative variation taking place within the microparasitic and host populations, and the variations in conditions which affect their interrelationships with each other and

infection may occur without demonstrable reaction on the part of the host, i.e., a symbiotic or a saprophytic relationship, but there is a difference of opinion as to whether the word infection should be used to describe such a condition

INFECTIOUS PERIOD

In clinical medicine, interest is centered upon a patient during the period that he or she is more or less incapacitated by the disturbance of physiologic functions caused by the invasion of a pathogenic micro-organism, that is, from the onset of symptoms to clinical recovery or to a fatal issue In epidemiology, interest must be broadened to include the whole duration of the host-parasite relationship, that is, from the time of the infective exposure until the microparasite is suppressed or eliminated from the host's body Of particular importance is the *infectious period*, the time or times during which the microparasite progeny are making an exit or are potentially available for transfer to a new host

CARRIERS

As early as 1890, Escherich noted that the infectious period of diphtheria was not necessarily coincident with the clinical course but that diphtheria bacilli might persist in the throats of patients during convalescence In 1892, Guttmann, Rommelaere and Simonds noted that cholera vibrios might be recovered from the feces during convalescence Credit probably belongs to Koch (Winslow, 1943) for grasping the important fact that cases which could be diagnosed clinically were not alone responsible for the spread of contagious diseases In his studies of cholera in Germany, during the winter of 1892-93, he noted that some cases were so mild that they escaped recognition and indeed could be detected only with the aid of a bacteriologic investigation The term "carrier" thus includes two classes. First, there are those who are about to have, or have already had, a clinical attack; they are sometimes designated as *incubatory*, *convalescent* or *chronic* carriers. Second, there are those who are suffering from a subclinical or asymptomatic infection, the so-called *healthy* carriers It is important to distinguish between these two classes, and for the purposes of this discussion, the second class of carriers are included in the designation *subclinical* or *inapparent* infections.

PATHOGENICITY

The characteristics of a clinical reaction and the ultimate issue of an infectious process in death or recovery are determined by the balance between the devices of aggression of a specific species of microparasite and the mechanisms of defense of the host species, potentialities for both of which are transmitted genetically The technical detail involved in classifying an individual as susceptible or immune is developed by what is known of pathogenesis and immunology.

The pathogenicity of a microparasite species for a human host population cannot be measured experimentally in animals It is indicated only by observations on the experience of *human beings exposed to a particular infection* under natural conditions To the extent that infection may result in recovery or death, pathogenicity is roughly indicated by the proportion of attacks which are fatal Stated in different words, it is a ratio between cases and deaths (the percentage of cases which are fatal) or the *case-fatality ratio* of a disease This ratio may be affected in a considerable measure by nonspecific conditions which affect the host population, such as starvation, lack of proper medical care, secondary invasion by other micro-organisms, and other factors To the extent that infection may result in a residual of impaired function, pathogenicity may be indicated by the proportion of cases which exhibit paralysis or other complications Finally, and more important, it is possible, in dealing with many infectious diseases, to estimate the proportion of persons infected with a particular microparasite who manifest a characteristic clinical reaction to those who do not, *the ratio of clinical to subclinical infections*. For example, in measles it is of the order of 19.1; on the other hand, in poliomyelitis it has been estimated to be of the order of 1:100 Such ratios vary not

(1) A cause-specific death rate or mortality rate:

$$\frac{\text{Number of deaths from a specific disease during a calendar period}}{\text{Average population present during same calendar period}} \times 100\,^{*}$$

(2) A case rate, attack rate or morbidity rate.

$$\frac{\text{Number of cases of a specific disease developing during a calendar period}}{\text{Average population present during same calendar period}} \times 100\,^{*}$$

(3) Prevalence ratio

$$\frac{\text{Number of cases of a specific disease existing at a particular time}}{\text{Population present or surveyed at that time}} \times 100\,^{*}$$

(4) Incidence rate

$$\frac{\text{Number of events in the population at risk during the specified time}}{\text{Mean population at risk of event during specified time}}$$

deaths, cases or infections (numerator) were discovered and recorded, and the clinical and laboratory criteria employed in diagnosis and classification The soundness of inferences drawn from biostatistical material can never exceed the level of accuracy of the original data

To represent [illegible] in [illegible] a h[illegible] hap[illegible] periods of time For adequate expression, the numerator of the fraction then should preferably be the number of *new* cases or *new* infections which are reported, or have their onset, or are discovered or are admitted to a clinic in successive days, weeks, months or years If the number of host population (denominator) remains relatively stable during the period under consideration, the number of new cases or new infections alone will suffice to indicate the course of events without calculating rates Thus the *incidence* of disease, or an *incidence rate*, is a dynamic concept It reflects changes in the frequency with which the microparasite is spreading and gaining access to new susceptible individuals, and, accordingly, the increase or decrease in microparasitic population

An accurate statement of incidence must take into account not only the number of new cases (numerator) but the total number of new individuals at risk (denominator) in each successive time period It makes a great deal of difference whether it is a closed or an open universe, i e , whether the population is composed of the same, or approximately the same, individuals throughout the period of observation or whether the individuals in the population are changing through immigration and emigration For example, an incidence of cerebrospinal meningitis in one army camp 10 times greater than in another when expressed on the basis of "cases per thousand strength per year" may be due to the fact that in the latter the personnel is permanent, while in the former it is changing periodically through the arrival of recruits and the departure of graduates from a course of training, so that 10 times as many individuals are at risk of infection during the course of a year.

A prevalence ratio (3) is, properly speaking, a static concept It represents how much

* This rate may be expressed on the basis of any population unit considered to be appropriate—per cent, per 1,000, per 10,000, per 100,000 The time unit chosen, whether it be hours, days, weeks, months, or years, is also varied according to circumstances

with the environment, results in quantitative changes The size of the microparasitic population depends upon the rapidity of passage from person to person and the accumulated proportion of persons harboring the infectious agent at any one time. This is determined not only by the proportion of susceptibles but by the opportunities for progressive transfer to new hosts, i e , the *exposure* or *contact rate* This rate is affected by a variety of conditions, *depending upon the requirements for transmission* For those diseases which are transmitted from person to person by some form of direct or indirect contact, the importance of the degree of crowding, or density of population, as determined by living in urban or rural areas, in private homes, in institutions, or in military installations, is obvious For those diseases which are transmitted to some extent at least by contamination of food, milk or water, the importance of sanitary conditions and home hygiene is evident For diseases transmitted by insects there are a whole series of conditions which affect the numbers of the vector species, their access to man, and requirements of the microparasite for completing a cycle of development. In every community the ecology is changing constantly with the habits of the people, day in and day out, from season to season, and from year to year

OPERATION OF CHANCE

If an individual in the infectious stage of a disease arrives in a community from which the disease has been absent for some time, what happens will be determined in part by the susceptibility status, in part by exposure or contact rates, and finally by the operation of chance For example, a person may develop measles and, since by chance the contacts immediately exposed are immune, no secondary cases will occur. Or, a second and a third case may occur without further transmission of the disease to susceptibles So the chain of propagation of an infectious agent may *build up or diminish and disappear*, depending on the one hand upon the continuing chance contacts between cases and susceptibles and on the other upon contacts between cases and immunes.

INCIDENCE OR PREVALENCE

The forces which create the dynamic biologic *phenomena of infectious disease are*, in the ultimate analysis, population pressures, i e., the innate impulse of living micro-organisms to multiply and survive by parasitism upon *homo sapiens* and the efforts of the host species to preserve its own integrity The balance between these two forces is fluctuating constantly, just as are the interactions between other living species, as for example, between the carnivores and their herbivorous food sources. When the equilibrium is a relatively stable one, it is manifested by an *endemic* prevalence. When the equilibrium is subject to sudden and violent disturbances, it is manifested by *epidemics* If the balance is in favor of the host, the disease shows a downward trend and tends to disappear If the balance is in favor of the microparasite, the disease tends to increase in prevalence and in certain instances may act as a temporary human population check if it is widely distributed and highly fatal

To facilitate reasoning, it is necessary to express these phenomena in quantitative terms. The basic elements of this statistical methodology are formulae which represent prevalence or incidence. The denominator (number of individuals in the host population) can in many situations be counted or estimated with considerable accuracy. The numerator (number of parasitic micro-organisms) can only be represented indirectly. It is correlated in a rough way with the number of deaths, or cases, or infections caused by a microparasite population. The indices schematically represented on the facing page are used most commonly

Obviously, each of the 4 types of rate has its own implications The one used will depend upon the questions to be answered and the availability of statistical information for the population group or groups under consideration. All are subject to errors of diagnosis and completeness of counting. Basic to effective use in reasoning is an assessment of the approximate validity of a rate. This can be done only when the accompanying text contains a clear statement of the universe of observation (denominator) in place or area, persons and time, the methods by which the

(1) A cause-specific death rate or mortality rate:

$$\frac{\text{Number of deaths from a specific disease during a calendar period}}{\text{Average population present during same calendar period}} \times 100\,^{*}$$

(2) A case rate, attack rate or morbidity rate:

$$\frac{\text{Number of cases of a specific disease developing during a calendar period}}{\text{Average population present during same calendar period}} \times 100\,^{*}$$

(3) Prevalence ratio:

$$\frac{\text{Number of cases of a specific disease existing at a particular time}}{\text{Population present or surveyed at that time}} \times 100\,^{*}$$

(4) Incidence rate

$$\frac{\text{Number of events in the population at risk during the specified time}}{\text{Mean population at risk of event during specified time}}$$

deaths, cases or infections (numerator) were discovered and recorded and the clinical and laboratory criteria employed in diagnosis and classification The soundness of inferences drawn from biostatistical material can never exceed the level of accuracy of the original data

To represent the *shift in balance* or *changes in equilibrium* between a microparasitic and a host population, it is necessary to show what happens in successive periods of time For adequate expression, the numerator of the fraction then should preferably be the number of *new* cases or *new* infections which are reported, or have their onset, or are discovered or are admitted to a clinic in successive days, weeks, months or years If the number of host population (denominator) remains relatively stable during the period under consideration, the number of new cases or new infections alone will suffice to indicate the course of events without calculating rates Thus the *incidence* of disease or an *incidence rate*, is a dynamic concept It reflects changes in the frequency with which the microparasite is spreading and gaining access to new susceptible individuals, and, accordingly, the increase or decrease in microparasitic population

An accurate statement of incidence must take into account not only the number of new cases (numerator) but the total number of new individuals at risk (denominator) in each successive time period It makes a great deal of difference whether it is a closed or an open universe, i e., whether the population is composed of the same, or approximately the same, individuals throughout the period of observation or whether the individuals in the population are changing through immigration and emigration For example, an incidence of cerebrospinal meningitis in one army camp 10 times greater than in another when expressed on the basis of "cases per thousand strength per year" may be due to the fact that in the latter the personnel is permanent, while in the former it is changing periodically through the arrival of recruits and the departure of graduates from a course of training, so that 10 times as many individuals are at risk of infection during the course of a year

A prevalence ratio (3) is, properly speaking, a static concept It represents how much

* This rate may be expressed on the basis of any population unit considered to be appropriate—per cent, per 1,000, per 10,000, per 100,000 The time unit chosen, whether it be hours, days, weeks, months, or years, is also varied according to circumstances

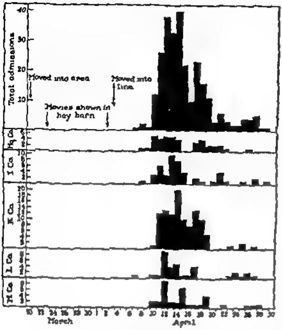

FIG 51 Outbreak of Q fever in the 3rd Battalion, *362nd Infantry*, indicated by daily hospital admissions for units as a whole and for each company (Adapted from Figs 2 and 3, Robbins, F C, Gauld, R L, and Warner, F B, 1946, A fever in the Mediterranean Area Report of its occurrence in Allied troops II Epidemiology, Am J Hyg *44*, 29)

disease there is in a given population existing at a particular time For example, it may be desirable to know how many cases of active tuberculosis, of primary syphilis or of rheumatic heart disease have been identified and are under medical observation in a particular community as of a certain date

COMMON-VEHICLE EPIDEMICS

The word "epidemic" is used most commonly to refer to the sudden or unusual appearance and/or temporary increase in incidence of a disease previously absent or occurring only sporadically in a particular population group and environment It is represented conventionally in a graph by plotting the number of cases (ordinates) by date of report or onset according to the selected time intervals, hours, days, weeks (abscissae) The numbers usually show a regular ratio of increase in successive intervals to reach a maximum and pass over into a similar ratio of decrease so as to describe a more or less symmetrical curve, as illustrated in Figure 51

It is to be noted that the span of time between the minimum and maximum incubation periods varies widely in different diseases For example, in food poisoning due to staphylococcus toxin it is a matter of 1 to 8 hours, in influenza from 1 to 2 days; in measles from 12 to 16 days, in homologous serum jaundice from 2 to 6 months By comparison of this span of time of the disease involved with the period during which the cases included in the outbreak have their onsets, an important inference can be drawn. If the onsets of all or nearly all of the cases fall within an interval no greater than that of the known variation in incubation periods, then it can be assumed that they arose from a nearly simultaneous exposure to a common vehicle of dissemination or to a single source Or, reversing the procedure, if it be known that the group of persons selected by a disease have been together upon only a single occasion, then the common exposure must have occurred at this time and the variation in the incubation periods of different individuals can be calculated. These considerations are illustrated by studies of an outbreak of Q fever among Allied troops in the Mediterranean Area, 1944-45. The following account is paraphrased from the report of Robbins, Gauld and Warner (1946).

In April, 1945, the 3rd Battalion, 362nd Infantry Regiment, with an approximate strength of 900, experienced an explosive outbreak of Q fever A total of 269 soldiers, or almost 30 per cent of the unit, were hospitalized between April 7 to 29, 1945, with an illness diagnosed as primary atypical pneumonia Early in March, the battalion was in the line where the various companies were widely dispersed On March 20 they moved back to rest and bivouacked in an area on the northeastern slope of a ridge about 0 5 mile from Paghana The 4 companies camped in a semicircle about a farmhouse in which the Battalion Headquarters was located and where the headquarters officers lived The farmer and his family remained in the house during this period. The men had pyramidal or pup tents for shelter, and some slept on the ground Some of them used hay from the barn adjacent to the house or straw from a

nearby haystack as bedding This bivouac area was occupied until April 3

While in this location an intensive training program was carried on which included the presentation of numerous training films and motion pictures These films were shown in the loft of the barn adjacent to the farmhouse, and attendance of all personnel in the battalion was compulsory The loft was large enough to accommodate one company at a time, and the companies attended in rotation

The course of the outbreak is shown in Figures 2 and 3 * The first patient was hospitalized on April 7, 4 days after the battalion had moved back into the line and 18 days after it first occupied the area near Pagliana The outbreak was explosive with the peak between April 12 and 14 On April 14 there were 40 hospital admissions from the 3rd Battalion By April 19 the outbreak was almost over, and the last patient was hospitalized on April 29 The total number of cases was 269, with 171 (63%) occurring in the 6 days from April 10 to 15

The occurrence of the cases, by company, is shown in Figure 3 † It will be seen that the outbreak occurred in all companies at about the same time

Only 4 cases of a similar clinical disease are known to have occurred in the other battalions of the regiment These 4 men were all members of the 2nd Battalion and all had attended the showing of training film in the hay barn in the 3rd Battalion area on the night of March 25 This was the only time 3 of them had been in the area, but the other patient was a frequent visitor

The explosive character of the outbreak would point to some common source of infection applicable to the entire battalion Water is unlikely as a source of infection because the unit's water supply came from an engineer point which supplied other units which had no disease

It would seem that the infection occurred during the period the battalion was bivouacked near Pagliana, since this was the only time the entire unit was brought together Assuming it to have occurred in this area, the time interval between possible exposure and onset of the outbreak fits exactly the incubation period previously estimated, 14 to 26 days In speculating upon the possible sources of infection associated with the area, suspicion immediately centered around the barn where the motion pictures were shown, particularly when one considers the 3 cases in men from the 2nd Battalion who had visited this barn

* See Figure 51, an adaptation of the figures mentioned here

† I e, in Robbins, Gauld and Warner

INVESTIGATION OF AN EPIDEMIC *

In undertaking such an investigation, it is desirable to have an orderly procedure The following outline is suggested as applicable, in general, to such situations

A Preliminary analysis on the basis of the information available at the time the investigation is begun

1 *Verify the diagnosis* This may require only a brief review of the clinical findings, or may necessitate getting laboratory tests under way

2 *Verify that an epidemic exists*, by comparing the incidence of the disease with its usual incidence in the community

3 Orient the epidemic as to *time* by determining the chronological distribution of dates of onset (the epidemic curve)

4 Orient as to *place* by determining the geographical distribution of cases

5 Orient as to *persons* by determining age, sex, race, and when possible, other characteristics of the cases, determine attack rates according to these characteristics

6 On the basis of a rapid preliminary analysis of the selection of the disease as to time, place and persons, formulate tentative hypotheses to guide further investigations Attempt to classify the epidemic as to mode of transmission as follows·

transmission by a common vehicle	single exposure continued exposure
propagated by	person-to-person spread arthropod vector animal reservoir

B Further investigation and analysis.

7 Search for additional cases which may not have been recognized or reported.

8 Determine what additional information is necessary to answer any questions formulated and to test tentative hypotheses Plan and conduct a detailed epidemiological investigation of all the cases (or of a representative sample of cases), using a suitable epidemiological case card Arrange for any special investigations needed to establish collateral cir-

* Maxcy, K F., 1956, Preventive Medicine and Public Health, ed 8, pp 1301-1302, New York, Appleton

cumstances, using laboratory facilities, engineering and other *expert consultation*

9 Analyze detailed data derived from case investigation, comparing attack rates among various pertinent groupings Try to identify the group selected for attack and discover the common source or vehicle *to which they were* exposed, if any Assemble results of collateral investigations.

10 Test various hypotheses which have been suggested to ascertain which one is consistent with all the known facts. Base conclusions on all pertinent [illegible] *upon any* [illegible] by itself [illegible] all the kr[illegible] until the s[illegible] evidence is consistent with only one hypothesis Formulate conclusions as to the source, *mode* of transmission and all other features of the epidemic which require explanation

A report of the investigation of an epidemic may be organized along the lines of the outline given above In addition, it should usually include a discussion of factors leading to the occurrence *of the epidemic, an evaluation* of the measures employed for its control, and *recommendations* for the prevention of similar episodes in the future

PROPAGATED EPIDEMICS

When the span *of time* of an epidemic wave is much greater than the average incubation period of the particular disease in question, then it can be assumed *that (1) exposure to* dissemination by a common vehicle has been prolonged, or that (2) the *infection is being* propagated by *progressive host-to-host transfer (contact transmission)*, or that (3) there *is a combination of common-vehicle dissemination* with *secondary contact transmission*

Frequently, the term "epidemic" refers only to a peak in the oscillating incidence of a disease more or less constantly prevalent in *a community*. How great *the increase of in*cidence must be before it is regarded as epidemic is a matter of judgment and is influenced by psychologic attitudes The greater the fear of a disease, or the more unusual it is *in a community*, the *smaller the increase* needed to justify use of the descriptive term Many statistical devices have been suggested for making the definition more objective and precise (Bundesen and Hedrich, 1925; Rich and *Terry, 1946*), but no definition has yet received general sanction. Dependence is placed in *general* upon comparing the current incidence of each specific infectious disease with its incidence in the past in the same population group and at the same time of the year. This *expected number* or *norm* is commonly expressed as a 3-year or 5-year *median of reported cases* When current incidence exceeds this number in several successive time periods, the disease shows a tendency which, *if sustained and great enough*, soon or late merits a pronouncement of the presence of an epidemic

Each infectious disease has a *seasonal* variation which follows a more or less regular pattern, *reaching a maximum distribution about* the same time each calendar year, when conditions are most favorable to transmission Each is *subject also to an interannual variation* or secular trend which may be slight or show wide fluctuations Some diseases manifest a cycle or *periodicity*, epidemic years occurring at fairly regular intervals of 2 or 3 years, or perhaps 4 or 5 years or *longer (Com*mission on Acute Respiratory Diseases, 1946) Others are entirely unpredictable in their annual behavior

EPIDEMIC THEORY

The simplest of all infectious diseases is measles *Table 10 (Wilson and Burke 1942,* 1943) illustrates the manner in which its incidence varies in any large city. By *progressive host-to-host transfer* the virus population maintains itself more or less continuously If it dies out completely, before *long it is rein*troduced by the importation of a case in the infective stage However, there is a rhythmical *variation in incidence* correlated with the season of the year increasing to a maximum in the spring and decreasing to a *minimum in* the summer months. The time at which the maximum incidence is reached in each year varies *within fairly wide limits* In some years, the total incidence is relatively low, in others it rises to a level regarded as epidemic These epidemic years appear to recur at fairly regular intervals in the same locality.

A century ago the periodicity of measles epidemics was known and discussed (Hirsch, 1883). The causes were thought to be obscure and complex, *although* it was generally

TABLE 10 MEASLES CASES BY MONTHS IN PROVIDENCE 1917-1940
(Adapted from Wilson and Burke, Proc N A S, 1943)

YEAR	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC.	TOTAL
1917	33	47	62	109	119	36	13	7	2	1	8	55	492
1918	35	98	373	1232	1299	780	261	23	8	6	5	3	4143
1919	1	4	4	4	5	4	3	3	1	2	1	3	35
1920	125	127	136	279	404	288	146	39	45	53	190	191	2022
1921	329	585	665	390	266	99	28	10	1	2	7	26	2408
1922	89	4	3	26	25	22	23	19	7	16	131	652	1017
1923	680	1228	1470	687	383	117	29	6	3	10	7	7	4627
1924	5	[illegible]	3	11	16	30	15	2	2	1	5	2	98
1925	13	11	6	15	18	30	58	50	13	81	417	1224	1936
1926	2057	1360	649	349	196	105	49	8	1	0	0	4	4775
1927	5	2	1	1	2	2	6	2	0	9	7	23	60
1928	45	112	422	1031	883	800	508	77	18	36	36	61	4079
1929	84	189	261	399	276	111	38	4	3	2	0	0	1367
1930	2	0	1	4	23	46	22	8	1	0	2	0	109
1931	1	2	49	158	456	358	179	99	22	191	337	1548	3400
1932	2799	2037	574	199	81	11	2	0	0	0	0	0	5703
1933	0	0	0	3	3	6	5	2	4	0	1	1	25
1934	4	11	21	18	29	106	44	23	8	5	1	7	279
1935	13	57	343	1351	1953	1279	241	17	4	1	0	48	5307
1936	119	74	92	76	83	17	11	4	0	0	9	77	562
1937	422	811	1184	711	472	129	31	4	0	2	3	3	3772
1938	2	5	4	2	0	0	0	3	1	0	0	3	[illegible]
1939	33	35	40	118	317	286	157	64	20	89	207	446	1872
1940	569	495	530	462	543	372	121	20	1	0	1	1	3115
Total	7483	7300	6892	7684	7852	4934	1989	495	165	507	1435	4385	51,223

Epidemics culminate in May, 1918, March, 1921, March 1923, January, 1926, April 1928, January, 1932, May, 1935 March, 1937, March(?), 1940 In this period of 262 months there are 9 major peaks, but we must not count both ends The average time between peaks is 33 ± 7.9 months, not 2 years For the mean we write 33 ± 2.8 months In Glasgow we estimate 40 months between peaks from 1888 to 1927, incl, based on Soper's data (*J Roy Statist Soc London*, 92, 34 61 [1929]) How many peaks one counts depends on the interpretation one gives to the qualifying adjective major and what allowance one makes for seasonal interruption of an epidemic

accepted that the accumulation of susceptibles was an important factor A more precise numerical approach to the explanation of periodicity of measles began with the contribution of Sir William Hamer (1906) Following his lead, a biometrician (Soper, 1929) in the course of an examination of possible methods of forecasting common contagious diseases "was led to adopt the simplest mathematical postulate that would describe on a first measure the generally accepted mechanism of epidemic measles, if the accumulation of susceptibles were really the prime factor, to compare the deduced results with the observed facts and then modify the primary hypothesis" Soper's work in turn stimulated to elaborate the statistical approach to epidemic theory. This has elucidated quantitatively the relationships of the principal factors involved, and contributed to a rational explanation of the epidemiologic behavior of measles

The fundamental facts with which one starts are simple The biologic attributes of the measles virus and the requirements for infective transmission from case to susceptible remain relatively constant The dynamics of the mass reaction are due to the flow of the virus through the human population Sus-

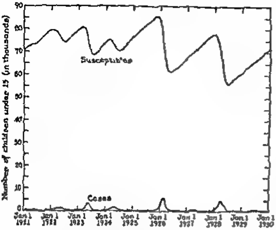

FIG 52 Secular trend of measles in Baltimore, Md, indicated by the estimated number of cases and susceptibles in the population under 15 years of age (Adapted from Fig. 3, Hedrich, A W., 1933, Monthly estimates of the child population "susceptible" to measles, 1900-1931 Am J Hyg. *17*, 626)

ceptibles effectively exposed to cases in turn become cases, cases recovering from the infection become immunes The susceptibles are being constantly recruited through birth and immigration, and depleted through becoming cases and immunes, or through deaths or emigration.

Upon the basis of a series of logical and reasonable approximations and assumptions, Hedrich (1933) made monthly estimates of the child population susceptible to measles in Baltimore from 1900 to 1931 As shown in Figure 52, during the 32-year period the calculated proportion of susceptibles in the population under age 15 did not rise above 53 per cent or fall below 32 per cent. The percentage figures are only approximations, but the implications are significant. When the proportion of susceptibles was low, the incidence of measles tended to be low, consequently, susceptibles accumulated When the proportion of accumulated susceptibles approached what McKendrick calls a *threshold density*, the situation was favorable for the support of an accelerated incidence of cases, or an epidemic. During a short period of time, the proportion of susceptibles fell rapidly as they became cases and subsequently immunes As the proportion of immunes increased, more and more cases failed by chance to make effective contact with susceptibles, and the incidence of new cases fell accordingly

It is apparent, therefore, that the principal factor determining the occurrence of propagated epidemics of measles is the proportion of susceptibles in the population at risk, and that the termination of an epidemic wave is due to the dampening effect of the cumulation of immunes and not necessarily to the complete exhaustion of susceptibles, since many escape effective exposure. The proportion of susceptibles required to support an epidemic, and per contra the postepidemic proportion remaining, will vary in every community, and even in the same community at different times of the year

By utilizing the simplified premises in measles, and representing the 4 principal factors by appropriate symbols, it is possible to derive a dynamic equation by which, given (1) the number of cases, (2) the number of susceptibles, (3) the number of the total population, and (4) assuming an arbitrary value for the *contact rates* in one time period of 14 days, the number of new cases which *will arise in the successive time* periods of the same length can be calculated In a community where sufficient data are available, the correspondence between cases predicated by such a formula and the cases observed is reasonably good within certain limits The same kind of reasoning and mathematical postulations can be applied to other infectious diseases However, the factors which go into the equation become more complex, and for an increasing proportion one is unable to obtain numerical values from observations made in nature The practical usefulness of the statistical approach to epidemic theory becomes correspondingly limited

EXPERIMENTAL EPIDEMIOLOGY

Another approach to the discovery of laws or general principles governing the behavior of infectious diseases in human populations is through observations made upon *epidemics in experimental animal colonies* Notable among the many contributions are those made by Theiler (1941) on mouse encephalomyelitis; by Traub (1939) upon choriomeningitis in mice; by Fenner (1948) upon mousepox (infectious ectromelia) of mice; by Webster

and his associates (1932, 1946) on salmonella, pasteurella, pneumococcus and Friedlander bacillus infections; by Topley and his associates (Greenwood, Hill, Topley and Wilson, 1936, Topley, 1942) on salmonella, pasteurella and ectromelia virus infections These studies are too extensive to permit detailed review.

The general procedure was to assemble uninfected animals in unit cages, the arrangement of which could be altered to simulate a community of any desired size. A constant regimen of cleaning and feeding was established, and appropriate measures taken to prevent the introduction of extraneous, pathogenic micro-organisms An epidemic was started by introducing into an uninfected animal colony a certain number of animals infected with the microparasite selected for the experiment The course of the subsequent epidemic was indicated by the occurrence of specific deaths, proved by necropsy and culture Effort was made to hold all of the important factors constant except the one under examination, and to note the effect this variable had upon the course of an artificially produced epidemic

It became evident very early in this work that a constant genetic stock of experimental animals was fundamental to control of the host variable As had long been known to the plant pathologists, it was found possible within certain limits by selective mating to breed out lines that were relatively resistant or relatively susceptible to infection with a particular micro-organism, such as *S enteriditis*, the cause of mouse typhoid It was demonstrated, for example, that there may be selected promptly from a hybrid stock of mice, of which 40 or 50 per cent die, lines in which as high as 95 per cent and as low as 15 per cent succumb following a standard dose of *S enteriditis* This afforded experimental evidence of the importance of innate differences in resistance genetically transmitted in human families, lines of descent, races, to a particular microparasite, a phenomenon well illustrated by the differences in the host reaction of the white and the Negro races to infection with *Myobacterium tuberculosis*

The possible importance of nutrition of the host to natural resistance to infection was appreciated If a diet were so poor in quality or quantity as to bring about a state of debility, experimental animals whose lives were already in jeopardy from the consequences of produced deficiency would have a higher death rate than well-nourished animals if subjected to the added insult of infection Obviously, it was desirable to hold this factor constant by providing a uniform and well-balanced diet It was noted, however, that a diet which was well balanced for normal growth and development was not necessarily well balanced in its effect upon host resistance to infection with a specific micro-organism This question has been explored extensively by many investigators in relation to various infections experimentally produced in animals It has been demonstrated that specific (natural) resistance can be influenced by nutrition when the stock is genetically heterogeneous and the pathogenic population to which it is exposed is heterogeneous in the sense that it contains an array of variation in terms of capacity to produce disease (Schneider, 1951). These studies have advanced the understanding of the underlying mechanisms implied in the terms resistance and susceptibility, but diet was of very limited importance as one of the variables affecting the results of experimental epidemics produced by *Salmonella enteriditis*

The variability in the biologic potentialities of the strains of infecting micro-organism employed received considerable attention A theory had been advanced by certain speculative epidemiologists that the rise and the fall of an epidemic, such as the pandemic of influenza, are due principally, if not wholly, to a progressive increase and decrease, respectively, in virulence of the specific agent, the increase being brought about by rapid passage of the infecting agent in human beings during the early part of an epidemic, and the decrease occurring because the infecting agent is subjected as an epidemic progresses to more resistance and less frequent passage as the result of increasing immunity in the host population To test this theory, methods were devised for measuring the virulence of a specific strain of micro-organism for groups of mice by administering a fixed dosage Sample cultures were obtained from animals dying at various times during artificially produced epi-

demics. Comparative titrations were made on strains from epidemics of pasteurellosis in rabbits, chickens and mice. Similar titrations of two serologic types were made during the course of mouse typhoid infections in mouse populations A total of 300 or 400 titrations were made under many conditions to test the theory of fluctuating virulence "The results were invariably negative and showed a constancy and fixity of disease-producing power of a given strain of organisms under all conditions of natural infection . . ."

From this experience, many were inclined to believe that in all instances changes in biologic potentialities of specific microparasitic species are of little or no importance in determining the rise and the fall of epidemic waves While this may be true within certain limits for many parasitic species, there are some which are more unstable and have considerable capacity for selective variation and adaptation in those qualities which determine its pathogenicity for a human population at a particular time and place. Reference is made here in particular to recent studies on the variability of viruses of influenza (Burnet, 1951) and of poliomyelitis (Sabin, 1951). The possibility that bacterial and viral dissociation may occasionally play a role cannot be ignored (Zinsser and Wilson, 1932). The development of sulfadiazine-resistant or penicillin-resistant strains of bacteria is a pertinent indication of what may happen in nature

In one series of studies it was demonstrated that, when infected animals were introduced in a closed universe of susceptible animals, the ensuing epidemic quickly subsided as susceptibles died or became immune, although some escaped infection An epidemic started in this manner could be maintained in an open universe if sufficient susceptible recruits were added at regular intervals If the conditions were held relatively constant, the balance between the microparasites and the host population tended to reach a stabilized equilibrium This was violently disturbed by a major change in the contact rate, which was accomplished by bringing a large number of animals previously dispersed in small single cages into a single colony in a large cage.

These and other experiments added support to some of the generalizations derived from experiences with epidemics in human populations under natural conditions They emphasized particularly the accelerating effect upon incidence of an inflow of susceptibles into an infected community, and of aggregation of individuals into large groups (crowding) and per contra the dampening effect upon incidence of accumulation of immunes. But the actual quantitative importance of each of these factors varies with the disease, its mode of transmission, the host relationships involved, and the local circumstances

EXTRAHUMAN RESERVOIRS

In the preceding paragraphs, for the sake of simplicity in discussing factors which determine incidence, attention was concentrated upon infections which are transmissible directly from one individual to another of the same host species. These are due to microparasites which in the process of host-wandering, mutation and selective adaptation have become so highly specialized in their nutritive requirements that they can grow and multiply only when enzyme systems of certain human tissues and cells are available to them. Other disease-producing agents are sufficiently plebeian in their nutritive requirements to be able to find conditions favorable for their propagation in selected organs and tissue cells not only of man but of other mammals, birds or arthropods.

Man, along with many other species of mammal, serves only as an aberrant host for the virus of rabies, which is dependent for its continuous propagation upon the canine species The virus of yellow fever can grow in the cells of many species of mammal, and in several species of mosquito found in the jungles of South America and Africa Primarily, the virus is native to jungle life, apparently principally dependent upon alternation of monkey and mosquito hosts Occasionally, it is transmitted to man, who, as an aberrant host, has "jungle" yellow fever, which is usually a sporadic disease But if an infected human being happens to reside in a community in which there are sufficient numbers of a domesticated species of mosquito, *Aedes aegypti*, and is bitten by them, they serve as efficient vectors in propagating an epidemic of yellow fever. Since the virus cannot make an effective exit from the human

host, except through the medium of blood-sucking mosquitoes, the disease is not otherwise transmissible directly from man to man

Rickettsiae are microparasites of arthropods. In the process of evolution they have become adapted to propagation in selected cells of man and other mammals. For example, *R tsutsugamushi*, the cause of scrub typhus, has established a symbiotic relationship with certain species of trombiculid mites (Blake, Maxcy, Sadusk, Kohls and Bell, 1945) Mites become infected in the larval stage, and rickettsial progeny are passed from generation to generation through the successive stages of development of the mite—nymph, adult, egg, larvae, etc This transovarial passage of the microparasite apparently does not interfere with normal growth, development and activity of the mite host Only in the larval stage does the mite seek a meal of tissue fluids from a mammal In the ecology of the mite vector, field rats are the most accessible source of such nutritive fluids In the process of feeding, the larval mite infects a rat, which suffers an inapparent infection While the rickettsiae are multiplying actively and being liberated into its peripheral circulation, the rat's tissue fluids are a medium of distribution to uninfected larval mites which happen to be feeding upon the animal at the time Thus the chain of transmission is maintained in nature without apparent detriment to either mite or rat population When an infected mite by chance feeds upon and infects a human being, the host reaction is manifested by the clinical signs, symptoms, and course of illness classified under medical terminology variously as tsutsugamushi disease, scrub typhus, mite typhus, etc Since larval mites have little or no opportunity to feed upon man during the stage of his illness when rickettsiae are in the peripheral circulation, and since the rickettsiae fail to make an effective exit from the human host in excretions or secretions, the infection is not passed directly from man to man

These illustrations serve to indicate the complexity of the factors which determine the incidence in human populations of diseases which are caused by microparasites with multiple host relationships The occurrence of human cases is the visible indication of the existence of an *extrahuman reservoir* There is some difference of opinion as to whether the term reservoir should be used to refer only to the principal mammalian or avian hosts or whether it should include arthropod hosts as well Rationally, it could be used with advantage to refer to the whole underlying extrahuman mechanism by which a specific microparasitic population is continuously maintained, including the specialized ecology necessary to support the biologic relationships involved

EVALUATION OF PREVENTIVE MEASURES

Knowledge of most of the common infectious diseases has advanced to a point where the principal factors which determine incidence and distribution are generally recognized However, with many, if not all, there is need for epidemiologic studies which will define more exactly these factors and establish their relative (crudely quantitative) importance. One may glibly state, for example, that measles is air-borne. However, it remains to be determined to what extent the virus is conveyed on particles, more or less indirectly by air currents from a case to susceptibles, the particle size which when inhaled will reach the mucous surfaces of the upper respiratory tract essential for infective contact, to what extent the virus is conveyed rather directly from person to person in what might be called conversational proximity, to what extent the virus is conveyed by contamination of articles with infective secretions and transferred by hands to the mouth of a susceptible, etc. The relative importance of these different routes of transmission must be evaluated if measures introduced to prevent spread are to be maximally effective To put the thought in more general terms, it is necessary to effectiveness that measures of prevention be directed against those conditions which are of actual importance in the particular situation rather than against the much wider range of conditions which may possibly contribute to the prevalence of a disease Innumerable instances could be cited in which public health campaigns or measures, thought to be theoretically sound and rationally conceived, failed to accomplish the reduction which was expected.

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has led in many instances in the past to the exploitation of biologic products and chemical substances which was unwarranted, and at times actually detrimental It has become painfully evident that evaluation by "clinical impressions" is unreliable

The basic requirements of field trials (Cockburn, 1957) upon human beings are well known, but the actual conduct of such an experiment is fraught with practical difficulties Ideally, two groups of persons a test and a control group, are placed under observation They must be alike in all essential respects, particularly those which relate to their susceptibility at the beginning of the experiment and their exposure to natural infection throughout the period of observation The substance to be tested must be administered without discrimination, if possible, alternate individuals should receive a placebo It is highly desirable that neither the subjects themselves nor the investigator who is responsible for their subsequent follow-up and observation should know who has received the test material and who has not In this manner, errors due to unconscious human bias may be obviated Individuals of both groups must be examined with equal frequency, care, and for equal periods, which are sufficiently long to ensure an adequate test of the protection afforded The criteria used in clinical diagnosis must be clearly stated The resulting attack rates in the two groups must be sufficiently large to be statistically significant

This is a basic outline of the kinds of problem encountered There are always many perplexing circumstances and occurrences tending to disturb the results for which allowance must be made in some manner

Illustration of this type of epidemiologic studies designed to evaluate critically an immunization procedure is found in the final report of a large-scale field trial of poliomyelitis vaccine (Salk) designed to evaluate protection afforded against paralytic attack (Francis et al, 1957) This study was comprised of two parts, conducted at the same time under a single plan for the collection of data, the so-called *placebo plan*, designed to assure strict comparability between the vaccinated and the control subjects This was accomplished by randomly selecting the two groups from a single volunteering population and by concealing the specific nature of the inoculum which each person received until all data were assembled and a final diagnosis made The *observed control plan* involved the vaccination of an identified segment of the children, while others were openly designated as the comparison group, without placebo The two studies involved different people in different areas and with different degrees of exposure to poliomyelitis The data for investigation were acquired from 211 different areas in 44 states and some from Canada and Finland They represent the results obtained with multiple lots of vaccine of varied potency used in areas where the nature and the degree of challenge differed widely

Intensive effort was made to assure completeness of registration, accurate records of inoculation, conformity with procedures for reporting of cases no matter what the prevalence of the disease, detailed, objective investigation of cases and their diagnostic classification under criteria considered by expert advisors to be valid within limits of diagnostic accuracy The data are highly reliable and complete The official study period was defined 2 weeks after the third injections were completed in a given area, corresponding to the time when specimens of serum were obtained for the measurement of antibody response The time was specifically determined for each area, but generally was about the middle of June To December 31, 1954, of the 1,012 cases considered to be poliomyelitis reported to the Vaccine Evaluation Center, in the total population of 1,829,816,428, or 57 per 100,000, developed in placebo areas, and 584, or 54 per 100,000, occurred in observed study areas.

REFERENCES

Blake, F G, Maxcy, K F, Sadusk, J F, Jr, Kohls, G M, and Bell, E J, 1945, Studies on tsutsugamushi disease (scrub typhus, mite-borne typhus) in New Guinea and adjacent islands, Am J Hyg, *41*, 243-373

Buck, C, 1956, Acute respiratory infections in families, Am J Hyg *63*, 1-12

Bundesen, H N, and Hedrich, A W, 1925, Method for early detection of epidemic trends, Am J Pub Health *15*, 289-296

Burnet, F M, 1951, Some biological implications of

SECONDARY ATTACK RATE

A classic example of critical evaluation of measures to prevent the spread of common contagious diseases is afforded in the development and the use of the secondary attack rate, with particular reference to scarlet fever and diphtheria, by Dr Charles V Chapin, for many years health officer of Providence, R I It is related in some detail by Frost (1938) in a discussion of the familial aggregation of infectious diseases

The principles and the applications of the methods have the merit of yielding information which is easily understood and directly related to the practical problems of the health officer The ultimate epidemiologic unit in a civil community is the family or household, a group of people (Buck, 1956) mostly of close kinship, sharing a common environment, living in close contact in a manner easily described, and usually under the eye of a single medical or lay observer The degree of contagiousness of different diseases can be measured by a statistical index derived from familial experience The first case to occur is designated as a primary or index case A census is made of the exposed members of the family, classified by age, sex, or other conditions which it is desired to take into account, especially with regard to their past history of having had the disease in question or specific immunization against it Then a record is kept of cases occurring in any member of the household within time limits, with reference to the index case, set specifically for each disease, so as to include those probably infected by contact It is then possible to summarize the observations on a large number of families and obtain an index of average experience based upon the ratio between secondary cases and exposed persons, or exposed persons specified as to age, sex, relationship, previous history, immunity status, or other quality However, it is to be noted that this index is based upon the frequency of secondary clinical cases following the occurrence of the index clinical case It does not take into consideration the spread by subclinical infections It is useful nonetheless in answering certain questions· for example, (1) given a case of a communicable disease in the family, what is the risk of *clinical attack* borne by others in the same household within specified periods of time? (2) to what extent can risk of clinical attack be reduced by preventive measures, such as sending the index case to the hospital or immunization of exposed susceptibles? It is pertinent to remark in this connection that a practical objective of preventive medicine is to decrease the risk of disease and death but not subclinical, immunizing infections

EVALUATION OF IMMUNIZATION AND CHEMOPROPHYLAXIS

The evaluation of preventive measures in reducing the incidence of a disease in a large population unit, such as a city, is fraught with difficulty Allowance must be made for the natural trend of the disease due to changes in complex factors other than those which are effected by the administrative measures Occasionally, nature performs an experiment, which if brought under adequate epidemiologic perception, answers a crucial question A classic example, which should be read by every student of epidemiology, is presented in the observations made by John Snow (1865) on the relation of the purification of water supplies to the incidence of cholera in different districts of London during the epidemic of 1854-55

However, nature seldom sets the stage for a scientific experiment in such a manner that it is possible to observe two population groups alike in all important respects except one So it becomes necessary to set up such groups artificially if many questions as to the effectiveness of control measures are to be answered.

The same kind of considerations enter into epidemiologic investigations designed to evaluate the prevention of an infectious disease by immunization or by the prophylactic administration of antibiotics or chemical compounds The preliminary work in testing effectiveness and safety is carried out in the *laboratory upon experimental animals,* where a susceptible species is available. When sufficient evidence has been accumulated to justify the use, the final evaluation of the efficacy of such agents can be obtained *only by human trial* Furthermore, these observations must be so controlled as to merit scientific acceptance of results Failure to meet this necessity

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12

Arthropod-Borne Animal Viruses

GENERAL CONSIDERATIONS

Arthropod-borne animal (arbor) viruses are defined as viruses which have the capacity to infect certain vertebrates—mammals and birds—and to multiply in the body of arthropods. Except under unusual circumstances, these viruses are not capable of spreading from vertebrate to vertebrate without the agency of an arthropod. The arthropod is the vector and becomes infected, generally, by ingesting blood from a vertebrate host at a time when the virus circulates in the latter. After a period of days, designated extrinsic incubation, the vector, by biting, can transmit the disease to a new susceptible host. Infection of the vector can also occur by transovarian transmission of the virus in the case of ticks. So far as is known, the virus does not cause any apparent ill effects on the vector, nor are any lesions detectable in the tissues of the arthropod.

It is the ecologic fact of the relationship between vertebrate and arthropod that brings together in a family all the viruses considered in this chapter; conclusive proof for support of the preceding definition is not at present available for all viruses concerned. In such cases, the virus is still included, either on the basis of serologic relationships with other agents belonging to the arbor family (for example, bat salivary gland virus), because the virus has been isolated from wild-caught arthropods (Anopheles A, Wyeomyia), or because it has been possible experimentally to infect arthropods and propagate the virus in them through several passages (Bwamba).

The explicit definition of an arbor virus, as multiplication in the arthropod without damage, excludes viruses which are transmitted mechanically by an arthropod, i.e., in the absence of multiplication in its body, and viruses which are intrinsic pathogens of arthropods. Finally, the term "animal" is included in the definition to distinguish these agents from viruses which are pathogens of plants and multiply in arthropods. The abbreviation arbor has been used because of its simplicity.

HISTORY

In the past 30 years nearly 50 distinct viruses have been found to be arthropod-borne, and continuing searches add new viruses to the group every year. Although they have been found on every continent, information on their geographic distribution is still fragmentary (American Geographical Society, 1952, 1954). The history of each virus, or of the diseases caused by them, is given in subsequent chapters. In this chapter the events are described that led to the establishment of the arbor family of viruses and its groups.

The crucial participation of arthropods, as vectors, in the dissemination of diseases caused by some viruses has been known for a long time. The first instance in which dependence

studies on influenza viruses, *Bull Johns Hopkins Hosp* 88, 119-180

Clark, E G, and Leavell, H R, 1953, Textbook of Preventive Medicine, New York, McGraw-Hill

Cockburn, W C, 1957, Field trials in the evaluation of vaccines, *Am J Pub Health* 47, 819-825

Commission on Acute Respiratory Diseases, Fort Bragg, N C, 1946, The periodicity of influenza, *Am J Hyg* 43, 29-37

Fenner, F, 1948, The epizootic behaviour of mouse-pox (infectious ectromelia) *Brit J Exper Path* 29, 69-91

Francis, T, Jr, et al, 1957, Final Report Evaluation of the 1954 Field Trial of Poliomyelitis Vaccine, Ann Arbor, Univ of Michigan

Frost, W H, 1927, Epidemiology, *in* Nelson Loose-leaf System, Preventive Medicine, Public Health Nelson, New York, vol 2, pp 163-190, *reprinted in* Maxcy, K F, Papers of Wade Hampton Frost New York, Commonwealth Fund, 1941, pp 493-542

———, 1938, The familial aggregation of infectious diseases, *Am J Pub Health* 28, 7-13

Greenwood, M, Hill, A B, Topley, W W C, and Wilson, J, 1936, Experimental epidemiology, Med Res Council, London, Special Reports, Series 209

Hamer, W H, 1906, The Milroy lectures on epidemic disease in England—the evidence of variability and of persistency of type (Lecture 3), *Lancet* 1, 733-739

Hedrich, A W, 1933, Monthly estimates of the child population "susceptible" to measles, 1900 1931, *Am J Hyg* 17, 613-636

Hirsch August, 1883, Handbook of Geographical and Historical Pathology, vol 1, London. The New Sydenham Society

McKendrick, A G, 1940, The dynamics of crowd infection, *Edinburgh M J* 47, 117-136

Maxcy, K F, 1941, Papers of Wade Hampton Frost, New York, Commonwealth Fund

Rich, W H, and Terry, M C, 1946, The industrial "control chart" applied to the study of epidemics, *Pub Health Rep* 61, 1501-1511

Robbins, F. C, Gauld, R L, and Warner, F. B, 1946, Q fever in the Mediterranean Area Report of its occurrence in Allied troops II Epidemiology, *Am J. Hyg* 44, 23-50

Sabin, A B, 1951, Paralytic consequences of poliomyelitis infection in different parts of the world and in different population groups, *Am J Pub Health* 41, 1215-1230

Sartwell, P E, 1950, The distribution of incubation periods of infectious disease, *Am J Hyg* 51, 310-318

Schneider, H A, 1951, Nutrition and resistance—susceptibility to infection, *Am J Trop. Med* 31, 174-182

Smith, Theobald, 1934, Parasitism and Disease, Princeton Univ Press

Snow, John, 1865, Snow on Cholera, New York, Commonwealth Fund

Soper, H E, 1929, The interpretation of periodicity in disease prevalence, *J Roy Stat Soc* 92, 34-61

Taylor, I, and Knowelden, J, 1957, Principles of Epidemiology, Boston, Little

Theiler, Max, 1941, Studies on poliomyelitis, *Medicine* 20, 443-462

Topley, W W. C, 1942, The biology of epidemics, *Proc Roy Soc, London, s B* 130, 337-359.

Traub, E, 1939, Epidemiology of lymphocytic choriomeningitis in a mouse stock observed for 4 years, *J Exper Med* 69, 801-817

Webster, L T, 1932, Experimental epidemiology, *Medicine* 11, 321-344

———, 1946, Experimental epidemiology, *Medicine* 25, 77-109

Wilson, E B, and Burke, M H, 1942, The epidemic curve, *Proc Nat Acad Sc* 28, 361-367

———, 1943, The epidemic curve II *Proc Nat Acad Sc* 29, 43-48

Winslow, C E A, 1943, The Conquest of Epidemic Disease, Princeton Univ Press

Zinsser, Hans, and Wilson, E B., 1932, Bacterial dissociation and a theory of the rise and decline of epidemic waves, *J Prevent Med* 6, 497-514

	St Louis	SLE	Muckenfuss, Armstrong, McCordock, 1933, Webster, Fite, 1933	+	+	+	+	Encephalitis	Mosquito	United States, Trinidad
	Uganda S		Dick, Haddow, 1952			+	+		Mosquito	Tanganyika, Uganda
	Wesselsbron		Weiss, Haig, Alexander, 1956	+	+	+	+	Influenzalike	Mosquito	South Africa
	West Nile	WN	Smithburn, Hughes, Burke, Paul, 1940	+	+	+	+	Denguelike	Mosquito	Egypt, India, Israel, Uganda
	Yellow Fever	YF	Stokes, Bauer, Hudson, 1928	+	+	+	+	Hepatitis	Mosquito	Equatorial Africa and America
	Zika		Dick, Kitchen, Haddow, 1952	+	+	+	+	Systemic	Mosquito	Nigeria, Uganda
	Diphasic Meningo Encephalitis, Diphasic Milk Fever		Smorodintsev et al , 1953, Chumakov et al , 1954	+	+	+	+	Diphasic Fever, Meningeal Signs	Tick	Northwestern USSR
	Kyasanur Forest Disease	KFD	Work, Trapido, Murthy, Rao, Bhatt, Kulkarni, 1957	+	+	+	+	Hemorrhagic Fever	Tick	India
	Louping-ill		Pool, Brownlee, Wilson, 1930	+	+	+	+	Encephalitis	Tick	*Great Britain, Czechoslovakia,* USSR (see Russian Spring-Summer Virus)
	Omsk Hemorrhagic Fever		Chumakov et al , 1947	+		+	+	Hemorrhagic Fever	Tick	USSR (Siberia, Great Barabin Steppes)
	Russian Spring-Summer Encephalitis	RSSE	Silber et al , 1937	+		+	+	Encephalitis	Tick	Austria, Czechoslovakia, Hungary, Malaya, Poland, USSR, Yugoslavia
C	Apeu		Causey, 1955		+				Mosquito	Brazil (Para)
	Marituba		Causey, 1955	+	+	+	+	Systemic	Mosquito	Brazil (Para)
	Oriboca		Causey, 1955	+	+		+	Systemic	Mosquito	Brazil (Para)
Ungrouped	Anopheles A		Roca Garcia, 1944			+			Mosquito	Colombia
	Anopheles B		Roca-Garcia, 1944			+			Mosquito	Colombia
	Bunyamwera		Smithburn, Haddow, Mahaffy, 1946	+		+	+		Mosquito	South Africa, Uganda
	Bwamba		Smithburn, Mahaffy, Paul, 1941	+			+	Systemic		Uganda
	California Encephalitis Virus		Hammon, Reeves, 1945			+	+	Encephalitis	Mosquito	Western United States
	Pongola		Kokernot, Smithburn, Weinbren, de Meillon, 1957			+	+		Mosquito	South Africa
	Rift Valley Fever		Daubney, Hudson, Garnham, 1931	+	+	+	+	Systemic	Mosquito	Kenya, South Africa, Uganda
	Simbu		Weinbren, Heymann, Kokernot, Paterson, 1957			+	+		Mosquito	South Africa
	Turlock		Lennette et al , 1957		+	+			Mosquito	Western United States
	Wyeomyia		Roca Garcia, 1944			+			Mosquito	Colombia
	Colorado Tick Fever		Florio, Stewart, Mugrage, 1944	+		+	+	Systemic	Tick	Western United States
	Crimean Hemorrhagic Fever		Chumakov, 1945	+		+	+	Hemorrhagic Fever	Tick	USSR (Crimea)
	Sandfly Fever—Naples Strain		Sabin, 1948	+				Systemic	Phlebotomus	Southern Italy
	Sandfly Fever—Sicilian Strain		Sabin, 1948	+			+	Systemic	Phlebotomus	Egypt, Sicily

TABLE 11 ARTHROPOD-BORNE ANIMAL VIRUSES (ARBOR VIRUSES)

Group	Virus	Abbreviation	First Reference to Virus and Name	Isolation in Nature from: Man	Isolation in Nature from: Other Vertebrates	Isolation in Nature from: Arthropods	Antibodies in Man	Human Disease in Most Severe Form	Vector, Suspected Vector or Isolations from	Isolations of Virus Reported from
A	Chikungunya		Ross, 1956	+		+	+	Denguelike	Mosquito	East Africa, South Africa
	Eastern Equine Encephalomyelitis	EEE	Ten Broeck, Merrill, 1933, Giltner, Shahan, 1933	+	+	+	+	Encephalitis	Mosquito	Brazil, Canada, Cuba, Dominican Republic, Eastern United States, Mexico, Panama, Philippines
	Mayaro		Anderson, Downs, Wattley, Ahin, Reese, 1957	+			+	Systemic		Bolivia, Brazil, Trinidad
	Middelburg		Kokernot, de Meillon, Paterson, Heymann, Smithburn, 1957			+	+		Mosquito	South Africa
	Semliki Forest		Smithburn, Haddow, 1944			+	+		Mosquito	East Africa, West Africa
	Sindbis		Taylor, 1953		+	+	+		Mosquito	Egypt, India, South Africa
	Venezuelan Equine Encephalomyelitis	VEE	Beck, Wyckoff, 1938, Kubes, Rios, 1939	+	+	+	+	Encephalitis, Influenzalike	Mosquito	Brazil, Colombia, Equador, Trinidad, Venezuela
	Western Equine Encephalomyelitis	WEE	Mayer, Haring, Howitt, 1931	+	+	+	+	Encephalitis	Mosquito	Argentina, Canada, Mexico, United States
B	Bat Salivary Gland Virus		Burns, Farinacci, 1956, Johnson, 1956		+					California, Texas
	Dengue Type 1		Sabin, 1952	+			+	Fever, Rash, Lymphadenopathy, Muscle and Joint Pains	Mosquito	Hawaii, India, Japan, Malaya, New Guinea
	Dengue Type 2		Sabin, 1952	+			+	As Dengue Type 1	Mosquito	India, New Guinea, Trinidad Uganda
	Ilheus		Laemmert, Hughes, 1947	+		+	+		Mosquito	Brazil, Guatemala, Honduras, Trinidad
	Japanese B Encephalitis	JBE	Kasahara et al., 1936, Kawamura et al., 1936, Taniguchi et al., 1936	+	+	+	+	Encephalitis	Mosquito	East Asian Mainland, Guam, India, Japan, Malaya, Ryukyus
	Murray Valley Encephalitis	MVE	French, 1952	+			+	Encephalitis	Mosquito	Australia, New Guinea
	Ntaya		Smithburn, Haddow, 1951			+	+		Mosquito	Uganda
	Spondweni		Kokernot, Smithburn, Muspratt, Hodgson, 1957			+	+		Mosquito	South Africa

constitute a definite serologic group. At least 14 additional viruses were excluded because their role in human infection is unclear and they have not yet been fully described. This is mentioned to emphasize the increasing number of new arbor agents that are being isolated. Arthropod-borne viruses of strictly veterinary interest were also excluded.

Basis for Classification. The present classification is based on serologic cross-reactions between viruses (Casals, 1957). All cross-reacting agents have been placed in a group; no virus has been found, so far, to show relationships with more than one group. Sera produced by repeated injections of a virus into an animal generally have shown a broader range of overlap within the group than sera produced by a single injection. Three different tests, CF, HI and NT, have been used for the detection of cross-reactions; with the better-studied groups, A and B, the HI test has given a broader spectrum of cross-reactions than the CF test, and the intracerebral NT test has been the more specific. With Group C, on the other hand, the CF test seems to be more inclusive, but even here the HI test has brought together all the viruses studied in the group. The classification shown in Table 11 derives from the results of HI tests with hyperimmune sera. Some viruses, particularly those of the Russian hemorrhagic fevers, have been classified on the basis of CF tests. Viruses that have cross-reacted in CF and NT tests have always done so within the group as established by HI tests.

As shown in Table 11, there are 3 groups of arbor viruses recognized, A, B and C. A considerable number of viruses that have not shown cross-reactions with any of the groups are left ungrouped. Possibly, in the future some of the latter may be joined to one of the established groups, or, since by NT and CF test reproducible cross-reactions have been found between some of the ungrouped viruses (Whitman, 1956; Casals, 1957), new groups may be identified.

Within Groups A and B, there are viruses which are more closely allied among themselves than to other agents in the group; these subgroups or complexes can be demonstrated by means of the less inclusive tests, CF and NT, or by the HI test using sera derived from experimental animals given a single injection of virus. These subgroups or complexes are:

Group A: (1) Chikungunya, Mayaro, Semliki Forest. (2) Sindbis, WEE.

Group B: (1) Dengue types 1 and 2. (2) Ilheus, JBE, Murray Valley encephalitis (MVE), SLE, WN. (3) Diphasic meningoencephalitis, Kyasanur Forest disease (KFD), Louping-ill, Omsk hemorrhagic fever, RSSE. The last subgroup is composed of agents so closely related serologically that it is questionable whether they are different entities. (4) Uganda S, YF, and possibly Zika.

The above classification omits entirely all other biologic properties of the viruses, i.e., host range, virulence by different routes of inoculation, length of incubation, type of disease produced in man and vector or arthropod from which it has been isolated, some of which may be important in their characterization. These properties are important, and characterization of a virus may be speeded con- [illegible]

classification is that, at present, the immunologic properties of a virus appear to lend themselves most readily to study and to be the most reproducible and stable from strain to strain.

Implications of Grouping. The existence of groups and the serologic overlaps on which they are based are considered as being due to antigenic constituents common to the related viruses. The associations in groups are of more than taxonomic significance; they are of importance in diagnosis and in cross-immunity (Casals, 1957). After inoculation of a virus into a fresh experimental animal, serum antibodies appear against the inoculated virus and against other viruses of the same group; the presence and the titer of the heterologous antibodies depend on the time between inoculation and bleeding, and on the virus inoculated. Inoculation of a virus into an animal previously injected with another virus from the same group provokes a general antibody response against all viruses of the group, which in breadth of overlap and in titer is far greater than the sum of the responses that each virus would elicit separately. In other words, a synergistic effect is demonstrable.

on a vector for the transmission of a virus infection of man was proved is yellow fever (YF) In early reports of the Yellow Fever Commission, Reed, Carroll and Agramonte (1901) gave conclusive proof that an arthropod vector, a mosquito, was essential for the natural cycle of the disease as it infects man. Doerr, Franz and Taussig (1909) investigated outbreaks of phlebotomus or sandfly fever and proved not only the filterability of the etiologic agent but also the fact that an arthropod vector was involved, in this case, a midge (Phlebotomus) Similarly, with another widespread disease, dengue, studies on its epidemiology conducted between 1906 and 1931 (see Chap 16), showed that in this disease too an arthropod vector, a mosquito, was involved Still another type of arthropod vector, a tick, was described by Gordon et al (1932) in connection with louping-ill; and, as reported by Silber and Soloviev (1946), a tick is also considered the vector of Russian spring-summer encephalitis (RSSE) In this disease, ticks are not only the vector, but they may well be a natural reservoir, as there seems to be evidence of transovarian transmission of the virus Finally, with more recent developments, emerged the recognition of the vector role played by arthropods, i e, mosquitoes, in the transmission of certain viruses which show neurotropic tendencies in clinical infections of man These diseases, e g, western equine encephalitis (WEE), St Louis (SLE) and Japanese B (JBE) encephalitis, were designated by Hammon and Reeves (1945) arthropod-borne viral encephalitides

In addition to the detection of ecologic or epidemiologic similarities, it has become recognized that many of the viruses in the arbor family fall into groups, the members of which are more or less closely related to each other and sharply separable from viruses belonging in other groups; this grouping has been made on the basis of antigen-antibody relationships detected by immunologic reactions

Interest in immunologic relationships began in the late 1930's, shortly after new agents, then designated "neurotropic," were discovered There are numerous reports in the literature that suspected serologic relationships between viruses could not be demonstrated, while subsequent work has shown them to exist. Advances in methods of study are in great part responsible for the detection of antigenic relationships Definite cross-reactions among 3 neurotropic viruses were detected by Smithburn (1942), who found by means of the neutralization (NT) test that West Nile (WN) virus and those of SLE and JBE were related Casals and Webster (1943, 1944) showed that the viruses of RSSE and of louping-ill were, by complement-fixation (CF) and NT test, closely related, if not identical Havens et al (1943) detected cross-reactions by CF test between eastern equine encephalomyelitis (EEE) and WEE viruses. The extensive use of the CF test for the investigation of cross-reactions has been of great value With this test, Casals (1944) confirmed the existence of common antigens among SLE, JBE and WN viruses, Hughes and Perlowagora (1950) found an overlap between SLE and Ilheus Additional cross-relationships were found by Sabin (1950), who was able to show, by CF test, that certain neurotropic viruses, JBE and WN and some non-neurotropic agents, dengue type 1, dengue type 2 and YF, were related Sweet et al (1953) reported cross-reactions by hemagglutination-inhibition (HI) test between SLE and dengue viruses In discussing these findings, Hammon (1948) and Theiler (1951) drew attention to the close similarity that a number of arbor viruses show in their epidemiologic patterns and ecology and the need for a general rather than an individual study of these agents. In a systematic study of a number of these viruses, using the hemagglutination (HA) and HI tests, Casals and Brown (1954) demonstrated extensive and sharply defined serologic relationships which permitted a number of arbor viruses to be assembled in two well-defined groups, designated A and B. Casals (1957), in extending this work, reported the existence of an additional group, C, and that there could be detected in Groups A and B subgroups, or complexes, of viruses closer among themselves than to the remaining agents of the group

CLASSIFICATION

In Table 11 are listed all the arbor viruses described in the literature A few soon to be described are included because there is evidence that they infect man or because they

constitute a definite serologic group. At least 14 additional viruses were excluded because their role in human infection is unclear and they have not yet been fully described. This is mentioned to emphasize the increasing number of new arbor agents that are being isolated. Arthropod-borne viruses of strictly veterinary interest were also excluded.

Basis for Classification. The present classification is based on serologic cross-reactions between viruses (Casals, 1957). All cross-reacting agents have been placed in a group; no virus has been found, so far, to show relationships with more than one group. Sera produced by repeated injections of a virus into an animal generally have shown a broader range of overlap within the group than sera produced by a single injection. Three different tests, CF, HI and NT, have been used for the detection of cross-reactions; with the better-studied groups, A and B, the HI test has given a broader spectrum of cross-reactions than the CF test, and the intracerebral NT test has been the more specific. With Group C, on the other hand, the CF test seems to be more inclusive, but even here the HI test has brought together all the viruses studied in the group. The classification shown in Table 11 derives from the results of HI tests with hyperimmune sera. Some viruses, particularly those of the Russian hemorrhagic fevers, have been classified on the basis of CF tests. Viruses that have cross-reacted in CF and NT tests have always done so within the group as established by HI tests.

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Within Groups A and B, there are viruses which are more closely allied among themselves than to other agents in the group; these subgroups or complexes can be demonstrated by means of the less inclusive tests, CF and NT, or by the HI test using sera derived from experimental animals given a single injection of virus. These subgroups or complexes are:

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The above classification omits entirely all other biologic properties of the viruses, i.e., host range, virulence by different routes of inoculation, length of incubation, type of disease produced in man and vector or arthropod from which it has been isolated, some of which may be important in their characterization. These properties are important, and characterization of a virus may be speeded considerably by preliminary investigation of some of its biologic properties other than serologic. The basic principle in proposing the above classification is that, at present, the immunologic properties of a virus appear to lend themselves most readily to study and to be the most reproducible and stable from strain to strain.

Implications of Grouping. The existence of groups and the serologic overlaps on which they are based are considered as being due to antigenic constituents common to the related viruses. The associations in groups are of more than taxonomic significance; they are of importance in diagnosis and in cross-immunity (Casals, 1957). After inoculation of a virus into a fresh experimental animal, serum antibodies appear against the inoculated virus and against other viruses of the same group; the presence and the titer of the heterologous antibodies depend on the time between inoculation and bleeding, and on the virus inoculated. Inoculation of a virus into an animal previously injected with another virus from the same group provokes a general antibody response against all viruses of the group, which in breadth of overlap and in titer is far greater than the sum of the responses that each virus would elicit separately. In other words, a synergistic effect is demonstrable.

That a synergistic effect also occurs in man can be deduced from studies following natural infections or experimental inoculations. Injection of dengue type 2 virus in persons previously vaccinated with YF (Schlesinger et al, 1956), injection of JBE virus vaccine in children convalescing from infection with SLE virus (Hammon et al, 1956); and inoculation of 17D yellow fever vaccine followed by WN virus (Price, 1957), resulted in marked serologic responses to the viruses inoculated and to others of the same group, B After natural infection with JBE, SLE or YF viruses (Casals, 1957), two types of serologic response were noted In persons with no serologically demonstrable previous contact with a Group B virus, infection was followed by a response in the convalescent serum specifically against the infecting virus by CF and NT, and nearly specific by HI; in persons with demonstrable previous contact with a Group B virus, infection was followed by a general antibody response to the group

Additional evidence of synergism in Group B was brought out in the course of serologic surveys (Theiler and Casals, 1957) Essentially, two types of response were manifested in the degree of specificity within the group and in the height of the titers, in one type, the serologic response was either specific or relatively specific in the HI test, nearly always completely specific in the CF, and even more so in the NT test, this was interpreted as pointing to infection with only one agent Another type of response was characterized by a broad serologic response at high titers to all Group B viruses in either HI or CF tests, even in the NT test cross-protection was evident. This was interpreted as pointing to superinfection.

The serologic overlap within a group plus the synergistic effect raise several important questions about diagnosis, immunization and epidemiology Serologic diagnosis of a natural infection in man is complicated by the cross-reactions. When confronted with a positive test, one must ascertain whether the result is due to homologous or heterologous antibodies (Sabin, 1950; Casals, 1957).

On challenge of immunized animals, relationships are found between representatives of this family of viruses Such crossings have been mainly between the more closely allied members of a group, particularly when the challenge was given by peripheral inoculation Thus, cross-protections occur between RSSE and louping-ill viruses (Casals, 1944); between JBE and MVE viruses (Pond et al, 1955), between WN, MVE, SLE and JBE viruses (Hammon and Sather, 1956); and in Group A, between Semliki Forest and Chikungunya viruses (Casals, 1957). Work with human volunteers showed cross-protection between dengue types 1 and 2 (Sabin, 1950, 1952) These observations, when added to the more general and inclusive concept of grouping, particularly in view of synergism, have suggested the possibility of protection against many viruses of one group through vaccination with only 2 or 3 viruses of that group (Schlesinger et al, 1956, Hammon et al, 1956; Price, 1957)

In connection with immunity against all viruses of a group, the following observations may be significant Monkeys or mice immune to Uganda S virus, when challenged with YF virus, showed a moderate resistance to the challenge (Macnamara, 1953). After injection of WN virus into chicks immune to JBE virus, the viremia was at a lower level than in nonimmune birds, mosquitoes feeding on birds inoculated with WN virus alone become infected and can transmit infection more readily than mosquitoes feeding on WN-inoculated birds previously immunized against JBE virus (Price, 1956) WN virus had a similar effect on the susceptibility of monkeys to JBE virus infection (Imam and Hammon, 1957) Of particular interest is the observation by Theiler (Chap 15) that sera from persons with neutralizing antibodies against one of several Group B viruses—dengue types 1 and 2, Ilheus, WN—protected mice against YF virus in a peripheral NT test; the same sera failed to protect mice in the standard intracerebral NT test. The implication is that infection with a Group B virus other than YF had altered the host-immune status, with the result that under certain conditions his serum could neutralize the YF virus. All these observations raise the possibility of changed epidemiologic patterns in a human population previously exposed to a virus when a second virus of the same group is introduced. However, it cannot be ignored that viruses of a single group are known to coexist in the

same area, and clinical cases have occurred in persons who already had antibodies to another virus in the group

GENERAL CLINICAL PICTURE

Man can react to an arbor virus in different ways In their mildest form, human infections result in sufficient multiplication of the agent to bring about formation of antibodies but no clinical manifestations, i.e., inapparent infection In somewhat more severe infection, a virus may produce a mild generalized illness and antibodies without evidence of localization, i.e., atypical or aberrant infection. In severe infection, the virus may cause characteristic syndromes, i.e., typical or fully developed disease A well-defined syndrome is probably the least common manifestation of infection, because antibodies are so often found in the serum of persons who have not had such a syndrome In certain endemic areas varying proportions of normal persons with no history of encephalitis have antibodies to EEE, Venezuelan equine encephalomyelitis (VEE), WEE, JBE, MVE or SLE viruses (cf Casals, 1958, Chaps 13 and 14)

The fully developed diseases that these viruses cause are varied Details of the clinical disease are given in the following chapters, and only the more salient general characteristics are listed in Table 11 It is worthy of mention here that with many arbor viruses human infection presents a diphasic course The symptom complexes do not necessarily follow the serologic groupings A number of the agents have caused no known disease in man, and the only indication of human infection is the presence of antibodies In the case of a few viruses, antibodies have not yet been found.

GENERAL PROPERTIES

The description of the properties of these viruses frequently refers to characteristics of a laboratory strain, and such properties as host range, pathogenicity for a given animal by different routes of inoculation, and capacity to produce an HA antigen may have changed Other properties, such as resistance to the action of physical or chemical agents and stability of virulence, may be affected by the concentration of virus in the preparation, by its purity or by substances in the medium that protect the virus.

Of all the easily measurable properties, those depending on antigenic constitution manifested in serologic reactions seem to be the most stable, even when other biologic properties are changed, as in the YF strains, Asibi and 17D, the serologic reactions remain unaltered. Since serologic reactions are specific for each virus, at least within quantitative limits, serologic reactions can be used for final identification of these agents

Physical and Chemical Properties. The reported sizes of these agents place them generally in the range of 15 to 40 millimicrons There are a few exceptions. Anopheles A and B, Bunyamwera, Bwamba, Ntaya and Wyeomyia have a particle size in the range from 70 to 122 millimicrons (Smithburn and Bugher, 1953)

In aqueous tissue emulsions these viruses are unstable at room temperature In the frozen state at temperatures such as -20° C all but a few of the viruses are more stable, but the titer drops progressively, and in several months to a few years virus infectivity may be lost In a solid carbon dioxide cabinet in a container sealed to exclude CO_2, the viruses are very stable, the titer remains virtually unchanged for years. There are exceptions with some viruses, usually linked with the nature of the diluent and the pH of the preparation

Lyophilization is a good method of preservation, although there may be an initial decrease in titer with some The protective action of normal serum and serum derivatives such as bovine plasma albumin is generally observed Advantage is taken of this property for preserving the viruses and for maintaining the titer of virus dilutions during animal inoculations

The pH of the suspension is important in maintaining the titer and the stability of virus dilutions during animal inoculation A pH in the alkaline range is preferred for most viruses, usually between 7 and 8 The pH influence on viral preparations is particularly noticeable in HA, where pH 9 is nearly indispensable to the preservation of the agglutinating property.

In most studies, the viruses have been

easily inactivated by the action of formaldehyde or ether, even in low concentration, this distinguishes them from others such as the poliomyelitis and the encephalomyocarditis Most of the agents are easily inactivated by exposure to ultraviolet light or by heating at 60° C. for 10 to 30 minutes.

An important property of the viruses is their behavior when acted on by sodium desoxycholate *Agents studied have been inactivated* completely by concentrations that do not affect the following poliomyelitis, Coxsackie, encephalomyocarditis and mouse encephalomyelitis (Theiler, 1957) The property is defined so sharply that it has been advocated for differentiation of this family However, susceptibility to the action of sodium desoxycholate is not an exclusive property of these viruses, it is also characteristic of agents of the influenza family and lymphocytic choriomeningitis, but it can be considered an excluding property in that no arbor virus has been shown to be resistant to this chemical. Another way in which arbor viruses *can be differentiated from intestinal viruses* is in their behavior toward ion-exchange resins. The conditions under which arbor viruses are adsorbed to the resin, Amberlite IRC-50, differ markedly from those required for adsorption of such viruses as poliomyelitis, Coxsackie, encephalomyocarditis and mouse encephalomyelitis (Clarke, D H, 1957, personal communication)

Biologic Properties. Host range in nature and pathogenicity for laboratory animals are of great importance Variability among strains has become particularly apparent in relation to susceptibility of laboratory animals; hence, most of the biologic properties described with respect to host range and pathogenicity refer to standard laboratory strains

Information on host range in nature depends on *many factors. For obvious reasons,* some viruses, such as YF and the encephalitides, have been investigated more extensively than others, and more ■ known about them Some *viruses, such as WEE are easily established* in common laboratory animals, while others, such as dengue types 1 and 2, are not; WEE has been recovered often in nature and in many species, while *the other two, even* though known to be widely disseminated, have been isolated infrequently. Finally, the time during which a virus may be isolated depends on its infective titer in tissues and the length of time it persists. Most attempts at isolation in man are confined to blood tests; therefore, if the *period of viremia is not long* or if it precedes symptoms, circulating virus can be missed These considerations make it understandable that the present knowledge of natural host range and distribution is incomplete There are viruses, i.e , dengue types 1 and 2 and sandfly fever, Sicilian and Naples strains, that have been isolated only from man, another agent, WEE, has been isolated from man, horse, squirrel, deer, birds, pig, mosquitoes, mites and a kissing bug Yet, the evidence of antibodies in man throughout the world indicates that the distribution of dengue encompasses the tropical zone, whereas WEE ■ far more limited in distribution

The *experimental host range* varies widely Some information on the susceptibility of laboratory animals is helpful in identifying certain of the agents Newborn mice have become a *standard test animal because they* are generally most susceptible; isolations from nature can be made in newborn mice which will fail in adults Further, the use of the newborn mouse for the preparation of *HA* and CF antigens has greatly improved the quality of these reagents

The newborn mouse is apparently the most universally susceptible experimental host. *All* agents listed in Table 11 are pathogenic for newborn mice on intracerebral inoculation, although all strains as they occur in nature may not necessarily be so. The pathogenicity of the viruses for newborn mice by peripheral inoculation or for adult mice by intracerebral or *peripheral inoculation has been inconstant;* strains have been adapted and propagated in adult mice which were not pathogenic for them at first.

The *developing chick embryo* is a valuable experimental host; some viruses have been originally isolated more easily in chick embryos than in newborn mice. However, it is *not as generally susceptible as the newborn* mouse

Propagation in tissue culture is another means of study. Many agents have been *propagated in Maitland type minced tissue* culture. With the success achieved by the

use of cell suspension cultures with poliomyelitis virus, many laboratories are investigating the application of these newer methods When suitable cell types are used, the viruses tested grow and multiply in tissue culture with variable degree of cytopathogenic effect (cf. Chaps 13 to 19).

In identification of newly isolated strains of established viruses, the determination of their pathogenicity for a few common laboratory animals can be of considerable help In such studies, by observing the pathogenicity for newborn and adult mice, chick embryos and 1-day-old chicks and the survival time after inoculation, good presumptive evidence may be obtained for identifying the strain (Whitman, 1956), but this must be confirmed by serologic study

Antigenic Properties The viruses yield CF and HA antigens that react in vitro Using as source material the infected brain tissue from newborn mice and a variety of procedures of extraction, CF antigens may be prepared for all tested viruses in the family except Group C In early passages of Group C, the brain tissue yields generally a poor antigen, but the liver tissue from infected newborn mice yields high titer CF antigens HA antigens have been prepared from either brain tissue or serum from infected newborn mice with all viruses in Groups A, B (no information on louping-ill or Russian hemorrhagic fevers) and C and also from ungrouped viruses, Bunyamwera, sandfly fever, Naples and Sicilian strains, and Rift Valley fever However, not all strains of a given virus have produced an HA antigen, in Group C, some agents not listed in Table 11 have failed entirely

DIAGNOSTIC METHODS

The details of serologic diagnosis are presented in Chapter 10 however, the problems encountered with arbor viruses will be reviewed in general Essentially, diagnosis consists of identifying the virus or the specific antibody The existence of group relationships makes it necessary to determine not only that a given virus or its antigens react with a given antibody but also that the reaction is homologous, not heterologous

To identify the virus, the first step is to prepare an immune serum against the agent and, at the same time, to prepare and test an antigen for its capacity to hemagglutinate erythrocytes. If the antigen is active, a test against known positive sera will determine promptly the group to which the virus belongs or its absence of group connection Often, this test alone will give a definite lead to the identity of the virus, unless one is dealing with a new agent If the preparation fails to agglutinate red cells, the group and even the closer relations within the group can be determined by testing the antigen by CF against a collection of stock positive sera Whether or not the group is determined at this stage, final identification requires testing with homologous immune serum When this is available, CF, HI and NT tests are carried out in which the isolate is set up against several known sera, and the serum for the isolate against several known viruses or their antigens In this manner, the homologous and the heterologous reactions determine whether the isolate is identical with a known agent. Occasionally, it is difficult to decide whether one is dealing with a new virus, closely related to another already known, or with a slightly different strain of a known virus As this situation has arisen repeatedly in recent years with JBE, MVE, RSSE, louping-ill and some of the Russian hemorrhagic fevers, to mention only a few, a type species could be conceived as a cluster of different varieties grouped around a prototype, rather than as strains identical with a prototype. Where one cluster ends and the next begins is not always clear A method which gives promise of providing a rapid and accurate way of surmounting some of the difficulties in identification of certain Group B viruses has been explored This consists in the use of standard antisera which have been adsorbed with a heterologous but related virus in order to remove all except the specific homologous antibodies, then an HA antigen derived from the virus under investigation can be characterized exactly by the identity of the specific serum that inhibits it (Clarke, D H, 1957, personal communication)

Regarding detection of antibodies, it is accepted that CF antibodies do not persist long after clinical infection, and little is known of their presence and persistence after sub-

easily inactivated by the action of formaldehyde or ether, even in low concentration; this distinguishes them from others such as the poliomyelitis and the encephalomyocarditis. Most of the agents are easily inactivated by exposure to ultraviolet light or by heating at 60° C for 10 to 30 minutes

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clinical intense viremia may be the most important sources of virus for vectors. A significant recent epidemiologic concept is that visibly healthy avian and mammalian hosts harbor the viruses and effectively provide them to vectors (Hammon, 1948, Theiler, 1951, Eklund, 1954).

The range of vertebrate species which may contract clinical infection or circulate virus in the blood stream is not known for every virus. Such information depends either on the experimental exposure and study of vertebrate hosts or on the detection of viremia under natural conditions.

Host-Vector Association. One or more arthropod species that can harbor and transmit the virus must associate closely with and prefer the blood of a vertebrate host receptive to the virus. The association must be close because the viremia is transient, and the vector is relatively short-lived. Each species of blood-sucking arthropod has an affinity for a vertebrate species or group of species. Detailed knowledge of vector-host preferences or dependencies is essential to the epidemiology of an arthropod-borne virus (Bates, 1949, Reeves, 1958). If the virus is to survive, the vector must have contact both with hosts in which viremia is pronounced and with susceptible hosts, in order to maintain the infection at epidemic or endemic levels. Earlier studies emphasized the relationship of suspected vectors to diseased human or animal hosts. This is an essential component of the epidemiologic pattern, but it may be more important to know how frequently vectors feed on vertebrate species having viremia without clinical symptoms. Even in studies of such classic infections as YF (Whitman, 1951), dengue (Smith, 1956) and the encephalitides (Ferguson, 1954), knowledge of host preferences or dependencies of primary vectors is limited. Detailed data on host preference from precipitin tests, bird malaria field epidemiologic studies and bait-trap studies illustrate the preference of primary vectors such as *Culex tarsalis* and *Culex univittatus* for the avian hosts of WEE, SLE and WN viruses (Reeves et al., 1944, 1954, Taylor et al., 1956, Dow et al., 1957).

In the laboratory many species of arthropods are effective vectors of the more extensively studied viruses, yet search for natural infection in endemic areas has failed to confirm their importance as vectors. Similarly, in many species of experimentally infected vertebrate hosts the virus may circulate in the blood, or the animal may be manifestly ill, yet evidence of their natural infection cannot be found, although they reside in endemic areas. Probably these efficient laboratory vectors do not usually feed on vertebrate hosts capable of biologic maintenance of the virus, and the effective hosts are not the blood source for efficient vectors in the field.

Under natural conditions the vector does not necessarily limit its blood feeding to the effective host. Characteristically, it will also feed on and transmit infection incidentally to species incapable of perpetuating the infection. Similarly, an effective vertebrate host will be bitten by other arthropod species incapable of perpetuating the infection.

Climate. Transmission depends on a climate favorable to completion of extrinsic incubation in the vector. Factors such as temperature, humidity and rainfall markedly influence the survival of the virus and the arthropod. The principal influences are on extrinsic incubation of the viruses, on the feeding activity or reproduction of the poikilothermic vector populations (Bates, 1949) and on the effect of physical environment on host populations.

If the vector is to transmit, during extrinsic incubation virus must pass from the lumen of the arthropod's intestinal tract to intracellular sites, multiply and reach the salivary glands in adequate quantity for an infectious dose to be inoculated by bite. The duration of the extrinsic incubation period is related inversely to temperature, being shortened by increased temperature. This undoubtedly reflects the effect of temperature on the metabolism of the poikilothermic vector and possibly on the virus. Epidemiologically, temperature is critical. When it is low the incubation is prolonged, and the cycles of transmission turn slowly. In temperate areas the arthropod-borne viruses are sometimes classified as spring-summer (RSSE), summer (WEE and SLE) or autumn-fall (JBE) infections. In part this marked seasonal limitation of clinical infections is dictated by the temperature requirements for extrinsic incubation. In contrast, in tropical areas temperatures may be

clinical attacks Hence, the CF test is not suited to surveys, but it is useful in diagnosis after clinical disease If compared with the NT test in surveys, the CF test probably would reflect the minimum rate of recent infection or perhaps a seasonal minimum infection rate In the CF, HI and NT tests, it is advisable to test each serum against several antigens or viruses. From a comparison of the respective titers of the serum against the different agents, a decision can be reached in most instances However, if a double or multiple infection with related viruses has occurred, interpretation may be difficult or impossible Neutralizing antibodies after clinical infection remain detectable for long periods, perhaps for life The duration of NT antibodies following subclinical infection is less well known The duration of HI antibodies has not been studied adequately, but apparently they persist considerably longer than CF antibodies Although the HI test is less specific in some groups than the NT test, the former is valuable in serologic surveys, particularly as a screening test for Group B antibodies in moderately to heavily infected endemic areas If the HI test alone does not answer the question of specificity, at least it selects positive and negative sera with considerable agreement with the results of NT tests The HI test separates positive sera from negative, while specificity is better shown by NT test.

EPIDEMIOLOGY

To understand the epidemiology of this family of viruses one must know their natural history in extrahuman hosts, because human infection generally is incidental to virus perpetuation. Studying the pattern of disease in human populations frequently assumes a position secondary to identifying the biologic circumstances on which the parasite depends for survival In the case of these viruses the usual epidemiologic approach is reversed· they are being recovered from arthropods before any accompanying infection or disease is identified in vertebrate hosts. Formerly, descriptive analysis of manifest infections led to identification of a virus and an arthropod vector

Transmission of virus from one vertebrate host to another depends on one or more arthropod species and will occur rarely, if at all, by other means such as contact between hosts. This dependence is proved or strongly implied by fairly constant association of the vector with the clinically or subclinically infected host and the absence of infection in the absence of the vector. Further, the virus can be isolated from a naturally infected vector species, as has WEE virus from *Culex tarsalis* or YF virus from *Haemagogus spegazzini*, and members of the species prove to be capable of transmitting the agent by biting an experimental host.

To learn whether all the necessary relationships exist between a host, a viral agent and an arthropod is a demanding task. In fact, the information is incomplete for most viral agents classified as arthropod-borne. Of the many types of blood-sucking insects and arachnids only 3 families, Culicidae, Psychodidae and Ixodidae, are clearly implicated as important biologic vectors of pathogenic viruses affecting man (Day and Bennetts, 1954, Reeves, 1958). The subsequent chapters present evidence that species such as *Culex tarsalis* for WEE, *Culex tarsalis* and *Culex pipiens-quinquefasciatus* for SLE, *Culex tritaeniorhynchus* for JBE, *Aedes aegypti*, *Aedes africanus* and *Haemagogus* sp for YF, and others are proved primary vectors

Biologic transmission consists of the following steps

1. A critical minimum amount of virus is necessary to establish infection in the arthropod vector

2 During extrinsic incubation in the vector, the period between ingestion and transmission by bite, the virus multiplies and reaches the salivary glands of the arthropod

3 The vector transfers virus to the vertebrate when it feeds on it

Vertebrate Host. One or more vertebrate species must be infectable by vector bite, and then virus must circulate in the blood stream in large amounts long enough to be provided to other vectors Biologically, this is the critical aspect of host-virus association The virus cannot be perpetuated if the vertebrate host cannot be infected by vector bite Characteristically, clinical manifestations need not accompany viremia, nor do clinical signs indicate previous or present intense viremia. Indeed, vertebrate species that support sub-

(Hammon, 1948; Reeves, 1958) and probably the dengue fevers (Smith, 1956) have adapted to new hosts and vectors By such adaptation they undoubtedly spread into geographic areas where their original hosts or vectors do not exist It is likely that this form of spread and subsequent host, vector or geographic isolation of the virus encourages antigenic variation from the parent strain of virus and in time results in a differentiable "type" This would explain the current differences and similarities of representatives within the A and B groups

One modification of the basic infection chain is characteristic of and unique, on the basis of present knowledge, to the tick-borne viruses (Silber and Soloviev, 1946, Florio and Miller, 1948) transmission of infection by female ticks through the egg to the subsequent generation This further assures survival of the virus

VERTEBRATE HOST
↓
TICK ─────┐
↓
TICK
↓
VERTEBRATE HOST ←┘
↓
etc

The implications of this adaptation are discussed in sections devoted to the tick-borne viruses (Chaps 14 and 18)

The preceding considerations of the factors controlling the life history of these viruses are essential to an understanding of the epidemiologic patterns of human infection

Epidemic Pattern. When infection is transmitted directly from man to man by an anthropophilic vector and the clinical attack rates are high, the epidemiologic characteristics and approach to study are similar in many ways to those of other epidemic virus diseases in a human population The cycle will generally be urban or domestic, because it depends on the availability and the close association of a large number of susceptible human hosts The infection will be epidemic and may involve all age groups Differences in susceptibility owing to age may distort the pattern if only overt infections are studied Continual transmission is essential to virus survival in the urban epidemic pattern Development of immunity by the host, decrease in the vector population or unfavorable climate may interrupt transmission

Variations in the virulence and the pathogenicity of these viruses may affect the epidemic pattern, as has been suspected and proved for other groups of viruses However, little is known of these characteristics of the arthropod-borne viruses in human infection under natural circumstances It remains for future studies to evaluate the epidemiologic significance of these virus characteristics

Human infection, immunity, population density and such factors are incidental to the survival of most arthropod-borne viruses, because they depend on other vertebrate hosts. A basic infection chain in lower animals may be active in areas with large human populations, even urban centers It may be diagrammed.

VERTEBRATE HOST
↓
VECTOR → MAN
↓
VERTEBRATE HOST

The entire proximal human population is at risk of being bitten incidentally by the infected vector If the human host does not have an adequate viremia or is not fed on by an effective vector, this chain reaches a "dead end" This type of cycle is exemplified by WEE, EEE, SLE, JBE, MVE, RSSE and Colorado tick fever Occasionally, some viruses might be transmitted from man to man several times and then die out because of limitations in host or vector On occasion there may be *contact infection between infected* animal hosts, as in VEE and EEE, or infection of man from contact with the infective discharges of animal hosts as in VEE Diphasic milk fever has adapted to animal hosts in such a way that transmission may be by arthropod vector or by milk

The epidemiologic pattern of incidental human infection with all the encephalitis viruses depends on the frequency of vector attack, the infectivity of the bite and the susceptibility of certain age groups to clinical infection Under optimal conditions for the virus, clinical attack rates of WEE, SLE and JBE may reach levels of 100 per 100,000 or above, even though the virus involved does

high enough for rapid extrinsic incubation and transmission (YF and dengue) in all seasons When these tropical viruses are introduced to temperate areas they may produce summer epidemics, but the onset of winter temperatures interrupts transmission. Having [illegible]

tribution of transmission by its direct effect on vector activities In temperate areas at low temperatures the vector may hibernate and interrupt or delay its feeding In the tropics during periods of prolonged high temperature and low rainfall the vector may go into a diapause phase and interrupt its feeding.

Levels of heat, humidity and rainfall affecting the reproductive cycle or longevity of vectors and vertebrate hosts will limit or increase the population and so decrease or accelerate transmission possibilities, through their influence on essential gross and microclimatic conditions Climate may even control the geographic distribution of the viruses as illustrated above.

Infection Chains. Bearing in mind the antigenic relationships of the arthropod-borne viruses and the biologic factors essential to their perpetuation, one suspects that phylogenetically they have evolved from relatively few prototypes The circumstances favoring such evolution may be clarified by diagramming a basic infection chain representative of the group and the possible variants

VERTEBRATE HOST
↓
VECTOR
↓
VERTEBRATE HOST
↓
VECTOR
↓
etc.

In the simplest model a single species of vertebrate host and of vector in close relationship maintain the virus All the epidemiologic factors of concern in other acute infectious diseases apply to the host, especially the number and the susceptibility of the population The vector replaces other means of transmission such as contact, mechanical vehicles, food or water. Transmission occurs if the virus is present, if there is a susceptible vertebrate population, if a large vector population effectively links the infective and susceptible host populations, and if the climate favors extrinsic incubation Examples of this type of infection chain are urban YF, dengue and phlebotomus fever.

Whether the infection is epidemic or endemic depends on the interaction of factors in the transmission cycle. It is immaterial to completion of the cycle whether infection in the vertebrate hosts is clinical or inapparent. The essential feature is that the infected host has sufficient viremia to infect vectors and that deaths or immunity responses do not reduce potential hosts to a level that interrupts the chain, causing the infection to disappear from the population.

Each virus survives in a basic infection chain In phlebotomus fever, epidemic dengue fever and urban YF, man is the vertebrate host, and *Phlebotomus pappataci* or *Aedes aegypti* are the only necessary vector species (Sabin, 1955, Taylor, 1951). However, one doubts that this is the basic infection chain for these viruses and suspects rather that the infection in man represents adaptation of the virus from a more basic chain, including a lower animal host and possibly another vector.

Probably man-to-man transmission of viruses by arthropods represents adaptation from another basic cycle, and they may also adapt to new vertebrate hosts other than man A new infection chain could develop if the vector were to transfer the virus to some other vertebrate host capable of supporting its survival Similarly, a new vector species may feed on the infected basic host and on subsequent feeding spread infection to its preferred host These possibilities may be diagrammed:

VERTEBRATE HOST	→	NEW VECTOR
↓		↓
VECTOR	→	NEW HOST
↓		↓
VERTEBRATE HOST		NEW VECTOR
↓		↓
etc		NEW HOST
		↓
		etc.

The viruses of YF (Theiler, 1951), certain encephalitides WEE, SLE, JBE and RSSE

Discovery of the true reservoir for one virus may clarify the problem with other members of the family.

Epidemiologic Tools and Procedures Epidemiologic study of the arthropod-borne viruses requires the services of a variety of professional specialists—physician, entomologist, virologist, vertebrate zoologist and veterinarian—and a well-organized virus laboratory

Proved clinical infections may be characterized in epidemiologic studies after diagnosis by virus isolation, serologic procedures and occasionally histopathologic or clinical procedures However, if studies are limited to clinical cases and epidemic periods, the true infection rate and basic transmission chain may remain obscure The extent of inapparent infection in man or other vertebrate hosts must be determined by serologic surveys for antibodies or tests for viremia Samples of arthropods must be tested for virus isolation to verify the vector species and their infection rates Frequently, findings must be confirmed by further study of the infection in vectors or hosts under controlled conditions

Current epidemiologic concepts have been formulated as a result of extensive application of these procedures and of detailed field studies of vectors and vertebrate hosts The development of control procedures for any of these virus infections must be based on knowledge of certain critical indices For the vector one would measure population, infection rate, infectivity rate by bite, and biting rates on vertebrate host species For host one would measure population, susceptibility and contact rate with the vector For climatic factors one would measure temperature and humidity and their effect on vector and host populations in the environment where transmission is expected

Control Control measures have been developed or considered for the relatively few infections of this group recognized as public health problems (Hammon, 1948, Smith, 1951; Sabin, 1955) Generally, efforts are directed either toward protection of susceptible hosts by immunization, as in YF, or toward vector control as in WEE, SLE, YF and dengue In addition, protection of individuals may be promoted by use of screens, repellents, or bed nets and isolation of clinical cases to prevent further vector infection All these measures may break vital links in the infection chain.

Immunization may be used selectively for members of a population under greatest risk of contact with vectors, or for an age group particularly susceptible to clinical infection Immunization has been used to protect man against YF and RSSE in rural circumstances and horses against WEE and EEE It can create a barrier if spread from person to person by vectors is feared, as in YF. However, for most of the viruses human immunization is still limited by lack of an effective vaccine, excessive cost, poor definition of the group at greatest risk, or ignorance of the period of greatest potential exposure (see Chaps 13 to 19)

Vector control is a promising approach if comprehensive epidemiologic study identifies the primary vector and proves it to be vulnerable to control and closely associated with man. In urban or domestic circumstances where a large human population is at risk of infection, reduction of the vector population may effectively interrupt transmission from person to person or reduce the risk of incidental spread of infection to man from a basic infection chain The feasibility of control may be limited by cost or by lack of knowledge of the vector's biology The ultimate effectiveness would be eradication of a vector from a geographic area, as is now being carried out for *Aedes aegypti* in areas of the Americas Under rural circumstances where man incidentally comes into contact with infected vectors, control may be impractical or impossible

Control through attack on the other vertebrate hosts in the basic chain has offered little promise for the few viruses with primary vertebrate hosts in a rural environment Inaccessibility of the hosts, economic or aesthetic value of the hosts and cost of control are prohibitive Conceivably in the future an ecologic approach may be developed in which the environment could be modified to control the host population

REFERENCES

American Geographical Society, 1952, World distribution of dengue and yellow fever (map), Atlas of Distribution of Diseases, plate 5

not depend on man for perpetuation. Clinical infections in man or other animals such as the horse are valuable indicators of virus activity, but as many infections remain subclinical the geographic extent of virus distribution cannot be measured solely on the recognition of clinical cases The basic infection chain may be active at such a low level and the vector attacks man so rarely that few or no human infections, clinical or subclinical, result This might best be referred to as a state of latent enzootic transmission

Endemic or Enzootic Pattern. Under endemic or more appropriately enzootic circumstances in rural areas, human infection may be limited to certain occupational, age or geographic groups, reflecting exclusive exposure of such groups or individuals to the vector in some other basic infection chain Man is usually a "dead end" in such a chain The circumstance seems to represent invasion by a susceptible host of an environment where risk of exposure to the vector is high Thus sporadic infection or disease may occur in middle-aged woodchoppers in endemic areas of YF (Taylor, 1951) or RSSE (Silber and Soloviev, 1946) and in agricultural workers in endemic areas of WEE (Longshore et al, 1956) Frequently, such cases are ascribed to a more common infection which produces similar symptoms.

Occasionally, endemic transmission occurs in urban or domestic centers where man is the only host In such circumstances the population is usually transient, with a constant immigration of susceptible people, or the majority of susceptibles are children in whom the disease is mild or atypical. A mixed susceptible and immune population where the vector population and climate are balanced may limit epidemic spread but is capable of supporting a smouldering endemicity (Taylor, 1951)

Reservoirs of Infection. A reservoir is a vertebrate or arthropod in which virus is maintained and can be disseminated for a prolonged period.

The tick-transmitted viruses have an effective reservoir because the vector is long-lived, remains infected for life and passes infection through the egg to at least a portion of its progeny. They can be maintained for long periods without vertebrate passage. In temperate areas the true "reservoir" status extends through winter. In contrast, vertebrate hosts of such viruses are infectious to ticks for only a few days.

Viruses transmitted by mosquitoes and sand flies have no apparent reservoir (Meyer, 1953; Reeves, 1958) The vectors may remain infective for life; but the adults are relatively short-lived except during hibernation or diapause, and there is no transovarian transmission In tropical areas a long-term reservoir may be unnecessary. Virus may persist by constant transmission whereby wandering epidemic or endemic centers maintain the contact of virus with nonimmune hosts and effective vectors. This idea has been challenged in recent years (Dick, 1953; Smith, 1956) The concept of wandering centers does not explain the persistence of virus in localities where a large proportion of hosts are immune and vectors are abundant, or persistence of infection through prolonged dry periods when vectors are few or inactive These circumstances in the tropics are comparable with temperate areas where viruses overwinter and show endemic persistence Possibly in temperate areas overwintering adult vectors maintain the virus, or occasionally vertebrate hosts have latent infections with intermittent viremia, or migratory hosts from more temperate areas reintroduce infection each summer (Hammon, 1948; Eklund, 1954, Reeves, 1958) Each possibility represents an extension in time or space of the basic infection chain

In current studies WEE and SLE viruses have persisted in experimentally infected *Culex tarsalis* through 3 winter months in a temperate area. The WEE-infected mosquitoes completed extrinsic incubation and could transmit infection (Bellamy et al, 1958). Both viruses have been isolated from naturally infected overwintering *Culex tarsalis* females during the winter and early spring when there was no evidence of active transmission (Reeves et al, 1958a, Blackmore and Winn, 1956). However, the pattern of virus occurrence indicated that latent virus infection in avian hosts was as likely to be the current source of vector infection as was the mosquito carrying infection through the winter WEE virus occasionally persisted in the organs of experimentally infected birds for 1 to 10 months (Reeves et al, 1958b).

alitis in the Yakima Valley, Washington, Am J Trop Med *24*, 131-134
Reeves, W C, Herold, R. C, Rosen, L, Brookman, B, and Hammon, W McD, 1954, Studies on avian malaria in vectors and hosts of encephalitis in Kern County, California II Infections in mosquito vectors Am J Trop Med *3*, 696-703
Reeves, W C, Hutson, G A, Bellamy, R E, and Scrivani, R P, 1958b, Chronic latent infections of birds with Western equine encephalomyelitis virus, Proc Soc Exper Biol & Med *97*, 733-736
Sabin, A B, 1950, The dengue group of viruses and its family relationships, Bact. Rev *14*, 225-232
———, 1952, Research on dengue during World War II, Am J Trop Med *1*, 30-50
———, 1955, Recent advances in our knowledge of dengue and sandfly fever, Am J Trop Med *4*, 198-207
Schlesinger, R W, Gordon, I, Frankel, J W, Winter, J W, Patterson, P R, and Dorrance, W R, 1956, Clinical and serologic response of man to immunization with attenuated dengue and yellow fever viruses, J Immunol *77*, 352-364
Silber, L A, and Soloviev, V D, 1946, Far Eastern tick-borne spring-summer (spring) encephalitis Am Rev Soviet Med, Special Supplement, pp 5-74
Smith, C E G, 1956, The history of dengue in tropical Asia and its probable relationship to the mosquito *Aedes aegypti*, J Trop Med *59*, 243-252
Smith, H H, 1951, Controlling yellow fever, *in* Yellow Fever, Strode, G K (ed), New York, McGraw-Hill, 710 pp
Smithburn, K C, 1942, Differentiation of the West Nile virus from the viruses of St Louis and Japanese B encephalitis, J Immunol *44*, 25-31
Smithburn, K C, and Bugher, J C, 1953, Ultrafiltration of recently isolated neurotropic viruses, J Bact *66*, 173-177
Sweet, B H, Chanock, R M, and Sabin, A B, 1953, Recovery and characterization of hemagglutinins from two immunologically distinct types of dengue virus, Fed Proc *12*, 462.
Taylor, R M, 1951, Epidemiology, *in* Yellow Fever, Strode, G K (ed), New York, McGraw-Hill, 710 pp
Taylor, R M, Work, T H, Hurlbut, H S, and Rizk, F, 1956, A study of the ecology of West Nile virus in Egypt, Am J Trop Med *5*, 579-620
Theiler, M, 1951, The virus, *in* Yellow Fever, Strode, G K (ed), New York, McGraw-Hill, 710 pp
———, 1957, Action of sodium desoxycholate on arthropod-borne viruses, Proc Soc Exper Biol & Med *96*, 380-382
Theiler, M, and Casals, J, 1957, Unpublished studies
Whitman, L, 1951, The arthropod vectors of yellow fever, *in* Yellow Fever, Strode, G K (ed), New York, McGraw-Hill, 710 pp
Whitman, L, 1956, Unpublished studies

American Geographical Society, 1954, Explored areas of arthropod-borne viral infections, yellow fever and dengue excepted (map), Atlas of Diseases, plate 13

Bates, M, 1949, The Natural History of Mosquitoes, New York, Macmillan, 379 pp

Bellamy, R E, Reeves, W C, and Scrivani, R P, 1958, Relationships of mosquito vectors to winter survival of encephalitis viruses II Under experimental conditions, Am J Hyg 67, 90-100

Blackmore, J S, and Winn, J F, 1956, A winter isolation of Western equine encephalitis virus from hibernating *Culex tarsalis* Coq, Proc Soc Exper Biol & Med 91, 146-148

Casals, J, 1944, Immunological relationships among central nervous system viruses, J Exper Med 79, 341-359

———, 1957, The arthropod-borne group of animal viruses, Tr New York Acad Sc (ser 2), 19, 219-235

———, 1958, Viral encephalitis, *in* Viral Encephalitis, Fields, W S, and Blattner, R J (eds), Springfield, Ill, Charles C Thomas, 225 pp

Casals, J, and Brown, L V, 1954, Hemagglutination with arthropod-borne viruses, J Exper. Med 99, 429-449

Casals, J, and Webster, L T, 1943, Close relation between Russian spring-summer encephalitis and louping-ill viruses, Science 97, 246-248

———, 1944, Relationship of the virus of louping-ill in sheep and the virus of Russian spring-summer encephalitis in man, J Exper Med 79, 45-63

Day, M F, and Bennetts, M J, 1954, A Review of Problems of Specificity in Arthropod Vectors of Plant and Animal Viruses Canberra Commonwealth Scientific and Industrial Research Organization, Division of Entomology, 171 pp

Dick, G W A, 1953, Epidemiological notes on some viruses isolated in Uganda, Tr Roy Soc Trop Med & Hyg 47, 13-43

Doerr, R, Franz, K, and Taussig, S, 1909, Das Papatacifieber, Leipzig und Wien, Deuticke

Dow, R P, Reeves, W C, and Bellamy, R E, 1957, Field tests of avian host preference of *Culex tarsalis* Coq, Am J Trop Med 6, 294 303

Eklund, C M, 1954, Mosquito-transmitted encephalitis viruses A review of their insect hosts and the mechanisms for survival and dispersion Exper Parasitol. 3, 285-305

Ferguson, F F, 1954, Biological factors in the transmission of American arthropod-borne virus encephalitides, a summary Public Health Monograph No 23, Washington, D C, Government Printing Office, 37 pp

Florio, L, and Miller, M S, 1948, Epidemiology of Colorado tick fever, Am J Pub Health 38, 211-213

Gordon, W S, Brownlee, A, Wilson, D R, and MacLeod, J, 1932, Studies in louping-ill, and encephalomyelitis of sheep, J Comp Path & Therap 45, 106-140

Hammon, W. McD, 1948, The arthropod-borne virus encephalitides, Am J Trop Med 28, 515-525

Hammon, W McD, and Reeves, W C, 1945, Recent advances in the epidemiology of the arthropod-borne virus encephalitides including certain exotic types, Am J Pub Health 35, 994-1004

Hammon, W McD, and Sather, G E, 1956, Immunity of hamsters to West Nile and Murray Valley viruses following immunization with St Louis and Japanese B, Proc Soc Exper Biol & Med 91, 521-524.

Hammon, W McD, Sather, G E, Lennette, E H, and Reeves, W C, 1956, Serological response to Japanese B encephalitis vaccine of children and horses immune to St Louis virus, Proc Soc Exper Biol & Med 91, 517-521

Havens, W P, Jr, Watson, D W, Green, R H, Lavin, G I, and Smadel, J E, 1943, Complement fixation with the neurotropic viruses, J Exper Med 77, 139-153

Hughes, T P, and Perlowagora, A, 1950, The antigenic relationships of certain viruses capable of producing encephalitis in mice, J Immunol 65, 155-161

Imam, I Z E, and Hammon, W McD, 1957, Challenge of monkeys with Japanese B virus after immunization by West Nile infection plus Japanese B vaccine, J Immunol 79, 253-258

Longshore, W A, Stevens, I M, Hollister, A C, Gittelsohn, A, and Lennette, E H, 1956, Epidemiologic observations on acute infectious encephalitis in California, with special reference to the 1952 outbreak, Am J Hyg 63, 69-86

Macnamara, F M, 1953, Uganda S and yellow fever viruses A slight relationship shown by experiments in Rhesus monkeys and white mice, Brit J Exper Path 34, 392-399

Meyer, K F, 1953, Vectors and reservoirs of virus diseases, Am J Trop Med 2, 757-770

Pond, W L, Russ, S B, Rogers N G, and Smadel, J E, 1955, Murray Valley encephalitis Its serological relationship to the Japanese-West Nile-St Louis encephalitis group of viruses, J Immunol 75, 78-83

Price, W H, 1956, The epidemic climate, Pub Health Rep 71, 125-135

———, 1957, Studies on the immunological overlap among certain arthropod-borne viruses, Proc Nat Acad Sc 43, 115-121

Reed, W, Carroll, J, and Agramonte, A, 1901, Experimental yellow fever Washington, D C, U S 61st Cong, 3rd Session, Yellow Fever, 1911, Senate Doc 822

Reeves, W C, 1958, Arthropods as vectors and reservoirs of animal pathogenic viruses, in Handbuch der Virusforschung, Doerr, R, and Hallauer, C (eds), Volume 4, Section 3, Hallauer, C, and Meyer, K F (eds), Vienna, Springer-Verlag, 688 pp

Reeves, W C, Bellamy, R E, and Scrivani, R P, 1958a, Relationships of mosquito vectors to winter survival of encephalitis viruses, I Under natural conditions, Am J Hyg 67, 78-89

Reeves, W C, and Hammon, W McD, 1944, Feeding habits of the proven and possible mosquito vectors of Western equine and St Louis enceph-

WESTERN EQUINE ENCEPHALITIS

(SYNONYMS FOR EQUINE ENCEPHALITIDES OR ENCEPHALOMYELITIDES *Die Amerikanische Encephalitis [beim Pferde]*, epizootic equine encephalomyelitis, WEE)

INTRODUCTION

Western equine encephalitis is observed as a summer viral infection of lower animals, causing disease in horses and mules The virus is transmissible to human beings in whom a malady is produced closely resembling that of St. Louis encephalitis, but in its antigenic behavior the virus more closely resembles that of Chikungunya, a denguelike disease The disease is distinct in several features from the maladies caused by infection with the eastern and the Venezuelan equine viruses

HISTORY

For more than 75 years, epizootics of encephalitis have been observed in equine animals in the United States Meyer et al, *1931*, isolated the causal agent from the CNS of affected horses in California by inoculation experiments in which horses, monkeys, rabbits, guinea pigs, rats and mice were used The agent is now known as the western equine encephalitis virus Howitt, *1938*, was the first to recover the virus from the CNS tissue and blood of man by means of intracerebral inoculation of mice The most extensive human epidemic occurred in 1941, chiefly in North Dakota, Minnesota and adjacent provinces of Canada, over 3,000 persons were attacked with a mortality rate of 8 to 15 per cent At the present time, there exists an endemic focus over most of the western United States and particularly along the Pacific coast For example, in 1945 and 1946, there were 57 and 149 cases, respectively, diagnosed as neurotropic virus infections at the Kern County (California) Hospital, of these, 18 and 9 patients, respectively, were definitely shown to have had western equine encephalitis, from 1947 to 1951, the incidence was still low In 1952, however, an explosive epidemic erupted in the Central Valley of California, of 813 cases diagnosed as infectious encephalitis, 375 were confirmed by laboratory tests as western equine and 45 as St Louis encephalitis, as many as 100 cases of the former and 16 of the latter occurred in Kern County (Longshore et al, 1956) In the United States, in 1954, 1,075 horses and mules were attacked and 357 died

CLINICAL PICTURE

The incubation period is usually from about 5 to 10 days but may be 4 to 21 days. The clinical picture varies considerably in different patients, from negligible signs and symptoms to acute comatose states developing within 24 hours

In the severe type, patients exhibit prodromata of headache, drowsiness, fever and gastro-intestinal disturbances, this is the systemic phase, in many the disease process stops here In others, it continues and suddenly, sometimes gradually fever appears with neurologic signs and symptoms which consist of severe headache, insomnia, and marked pain in the muscles, especially those of the back Lethargy, disturbances of speech, ataxia, nystagmus, tremor, convulsions, mental confusion, amnesia, and even coma may supervene Paralysis is not common, occurring in about 15 per cent of those attacked, ophthalmoplegia and ptosis are still more uncommon The acute phase endures from 7 to 10 days Most patients recover completely, residuals such as parkinsonism are rare Clinically, it cannot be differentiated from St Louis and other forms of infectious encephalitis Abortive forms may be seen, here some of the prodromal symptoms, such as fever and headache, may be the sole indications of infection Clinically inapparent cases, i e, those in which no obvious signs except development of serum antibody are discernible, frequently are observed during epidemic and interepidemic periods

The white blood cell count during the encephalitic phase exhibits, as a rule, a slight polymorphonuclear leukocytosis, the total count ranges from 10,000 to 16,000 The cerebrospinal fluid shows a pleocytosis of 10 to more than 400 cells; early, polymorphonuclear leukocytes are in evidence, but about [illegible]

in amount

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13

Arthropod-Borne Group A Virus Infections of Man

The history and the classification of the arbor viruses are given in the preceding chapter, the viruses of Group A and the diseases induced by them are discussed in this chapter. Certain concepts are defined that apply to this as well as to the other groups.

Of the several types of disease in most severe or typical form caused by arbor viruses (cf. Table 11, Chap. 12), denguelike, encephalitic and systemic syndromes are represented in Group A. Of these diseases, the encephalitides caused by western and eastern equine encephalitis viruses are of particular interest. They, as well as those caused by some Group B viruses (cf. Chap. 14), were designated by Hammon and Reeves, 1945, as "arthropod-borne viral encephalitides." This designation, proper when applied to the encephalitic syndrome, should not be extended to the causal virus itself. The concept that prevailed earlier, that the viral encephalitides were induced by agents designated as neurotropic (encephalitogenic) which attacked, as a rule, nervous tissue or neurons, has been gradually modified. In the natural history of the diseases and in the evolution of experimental infections, neurotropism has been found to depend on such variables as the species of the host, the strain of virus involved, its manipulation in the laboratory, and the route of inoculation in experimental infection. For example, the virus of yellow fever, which is viscerotropic in man in nature, can be made to exert a highly neurotropic action in mice; that of Venezuelan equine encephalomyelitis is neurotropic in laboratory animals but in man induces ordinarily a mild systemic infection. Yet, the encephalitides and the viscerotropic maladies and their viruses have many features in common, as was shown in the preceding chapter. One of the striking similarities is that the encephalitis can be considered generally as incidental or uncommon, and as will be shown later, the largest incidence of infections among the so-called "encephalitides" is clinically inapparent, abortive, or systemic.

The subject of human infections with arbor viruses, apart from yellow fever and dengue, has developed only during the last 25 years. From 1931 on, and in different parts of the world, approximately 50 viruses known or suspected of being arthropod borne have been discovered. Eight of these belong to Group A: western, eastern and Venezuelan equine encephalomyelitis, Semliki Forest, Sindbis, Mayaro, Chikungunya and Middelburg.

tissue with a sedimentation constant of 265 S or a diameter of 45 mμ; this component carried all of the infectivity. Studies on the chemical constitution of the infective particles purified by ultracentrifugation were reported by Beard, 1945, and colleagues (Sharp, 1953); the particle contained 54 per cent fat-solvent extractable material, 4 per cent carbohydrate and the remainder ribonucleoprotein. One should consider the limitations of a technic which may not yield a pure material (Sharp, 1953), the chemical results stated may need revision if more purified virus preparations become available. Electronmicrography of the virus is reported as revealing images resembling those of the active agent of papilloma, i e, round particles with ill-defined edges and internal structures, some dense, others vacuolated. The ultraviolet light adsorption spectrum of the purified virus is similar to that of nucleoprotein, inactivation of 10^6 infective units is reached in 13 minutes (lambda = 2,600 Angströms) at a radiation energy of 252 ergs/cm²/sec (Sharp, 1953). Ninety-nine per cent of the virus can be adsorbed on Willstaetter's type C aluminum hydroxide from which it can be eluted, Cox and Olitsky, 1934.

The maximum stability of the virus at icebox temperature is between pH 6 5 and 8 5, its infectivity is rapidly lost in suspensions more acid than pH 6 5. In suspensions, the virus withstands a temperature of 70° C, and in filtrates, a temperature of 60° C, for 10 minutes. In the preparation of vaccines, the virus is inactivated by keeping the suspension in the presence of 0 4 per cent formalin for 2 days at 20° C and then for 2 days at 4 or 5° C. It can be preserved in cold 50 per cent buffered glycerol at pH 7 4 or 7 5, by being held in the frozen state on dry ice, by lyophilization, or by being held in 2 per cent bovine serum albumin at −20 to −25° C, Olitsky et al, 1950, or in 60 per cent heat-inactivated rabbit serum, Olitsky et al, 1949.

The western virus grows readily in tissue cultures. Three findings with this virus as a model have led to important results. The first was made by Huang (1942, 1943) who observed cytopathogenesis in tissue cultures and its neutralization by immune serum which led to the development of improved technics for virus isolation and for antibody measurement (Syverton and Scherer, 1953; Fastier, 1954). The second finding was reported by Dulbecco (1952). In a monolayer of chick-embryo fibroblasts cultivated in vitro, plaques of necrotic areas appear, each presumably produced by one virus particle. This has become a useful technic for certain other viruses (Dulbecco, 1955; Dulbecco et al, 1956). The third finding was made by Trager (1938) who showed that this virus increases in titer when it is cultivated in the presence of mosquito tissue.

After natural or experimental infection, the virus induces a solid immunity, which can also be achieved by the use of vaccines consisting of virus inactivated by formalin as shown by Shahan and Giltner, 1934, Cox and Olitsky, 1936, Beard et al, 1940. Both complement-fixing (CF), virus-neutralizing (NT) and hemagglutination-inhibiting (HI) antibodies are associated with this immunity. Thus, in human beings ill with the infection, NT antibody is found within 7 days after the onset, it may persist for at least 12 years. CF antibody is also found within 7 days after the onset, but in the majority of cases it is not found 12 to 14 months after recovery, and HI antibody is first noted 1 to 7 days after onset of symptoms, much earlier than CF antibody. It is not yet known how long this persists. After vaccination of human beings, NT antibody lasts for at least 2 years, but little or no CF antibody is produced. In experimental animals after vaccination, NT antibody appears within 3 days and endures for at least 6½ months, CF antibody develops on the ninth day and lasts for at least 4 months, HI antibody is noted first within 7 days. The duration of antibody as stated is based on the longest time after vaccination at which tests were made.

Cox and Olitsky, 1936, and Morgan et al, 1942, demonstrated that mouse-brain vaccines can be used for the experimental production of solid and enduring immunity to challenge doses of the virus given intracerebrally and peripherally. In such vaccinated animals, there is correlation between the amount of NT antibody in the serum and the immunity against viral infection. This work points to the availability of antibody to the CNS as being an important factor in the manifestation of such

Pathologic Picture

Macroscopic and microscopic changes in the CNS closely resemble those of St Louis encephalitis and are essentially meningo-encephalitic, often the cord is not involved, although in some instances a few small lesions are seen in the upper cervical region As a rule, only a slight lymphocytic infiltration of the meninges occurs In the gray matter, there are widespread lesions consisting of focal accumulations of glial cells, perivascular lymphocytic infiltration, spongy disintegration of the ground substance, neuronal degeneration, and a varying degree of neuronal necrosis with neuronophagia A diffuse infiltration of polymorphonuclear and mononuclear cells may be observed The lesions are extensive in the gray matter, but scattered microglial aggregations and plaques of myelomalacia without a surrounding inflammatory reaction can be seen in the white matter Some blood vessels show an inflammatory reaction in their walls, and occasionally thrombi are seen

Experimental Infection; Host Range

The western virus has a very wide host range In nature, human beings, horses and mules have shown signs of infection, and the virus has been isolated, in addition, from *squirrels, deer, pigs and birds* The experimental infection with signs of illness can be induced by intracerebral inoculation in albino mice, hamsters, rats, guinea pigs, domestic and wild rabbits, monkeys, squirrels, cotton rats, kangaroo rats, wood rats, wild mice, young dogs, deer, pigs, gophers, calves, goats, prairie chickens and pigeons Sheep and cats are resistant Barnyard fowl and certain wild birds, after exposure to the virus by dermal inoculation, have a viremia but are otherwise unaffected (Hammon and Reeves, 1946, Hammon et al, *1951*) A viremia followed by CNS invasion is seen in the Syrian hamster which, in adulthood (*85* to 90 Gm.), is highly susceptible to peripheral (intramuscular, intraperitoneal, intracutaneous and subcutaneous) exposure *to the virus (de Boer et* al., 1955).

The animal of choice for many phases of experimental work is the white mouse. Old and young (15-day-old) mice are equally susceptible to the virus given intracerebrally and intranasally. Mouse-adapted virus, when introduced intracerebrally, produces disease in dilutions of 10^{-8} and rarely 10^{-10}; dilutions of 10^{-3} are effective by intranasal instillation From *2* to *6* days after inoculation, mice show signs of meningo-encephalomyelitis, e g, generalized spastic muscular contractions, spastic paralyses, wild or in-co-ordinated movements, torpor, prostration and death Death may ensue within a few hours after onset of signs or at any time up to *2* days later The pathologic picture simulates that observed in man After intraperitoneal or intramuscular inoculation of *15-day-old* mice, virus appears in the blood, and in the majority of instances, it migrates from the blood to the nasal mucosa, whence it invades the CNS by the olfactory route. Sabin and Olitsky, *1938*, showed that most old mice are resistant to the virus given by peripheral routes other than the nasal Newborn mice, on the other hand, are highly susceptible *to the* virus injected intraperitoneally *or subcutaneously, the titers being* similar *to those* obtained following intracerebral injection, Olitsky and Harford, 1938 With this virus, as well as with that of EEE, Hurst, 1936, reported that following infection of monkeys and guinea pigs, there is a visceral phase followed by a nervous one.

Chick embryos are highly susceptible to *this virus and* develop a characteristic rapidly lethal infection by all methods of inoculation, with titers between 10^{-7} and 10^{-9}. After inoculation by any route, the embryo dies within 18 *to 24* hours, showing widespread hemorrhage, thrombosis and necrosis throughout all tissues Half-day-old (not 1-day-old) chicks have been found to be highly susceptible to this and the eastern virus and are proposed *for use in* virus isolation *and* neutralization tests (Chamberlain, Sikes and Kissling, 1954).

Etiology

The diameter of the virus, estimated from results of filtration through gradocol membranes, is about *25* mμ; from electron microscopy and ultracentrifugation, about 40 mμ, by sedimentation, *57* mμ (Sharp, *1953*) It is filterable through Berkefeld V, N, and W and through Seitz filters Studies by ultracentrifugation on the virus grown in chick embryos, Beard, 1947, showed the presence of a component not present in the normal embryo

(1955a) also reported NT antibody in the plasma of domestic fowl The disease also occurs during the summer in Canada and the Argentine Republic, Causey and Theiler (1958) found NT antibody in 3 of 93 residents of the Amazon Valley, Brazil. In some outbreaks, victims of infection have frequently been adult males, 20 to 50 years of age, who work outdoors In the epidemic in Manitoba in 1941, a high attack rate was also noted in infants under 1 year of age. In the San Joaquin Valley in California, attack rates are highest in infants and children (Longshore et al, 1956) The mortality rate is said to be from 7 to 20 per cent, the average being about 10 per cent

There is solid evidence that the primary vector in the Western United States is a culicine mosquito, *Culex tarsalis* This mosquito has been found naturally infected in California, Washington, Nebraska, Montana, North Dakota, Minnesota, Colorado and Manitoba (Ferguson, 1954; Eklund, 1954), this mosquito is also a most efficient vector under experimental conditions (Hammon and Reeves, 1943, Barnett, 1956) and has a predilection for bird blood (Reeves and Hammon, 1944, Reeves et al, 1954) Birds, after infection by vector bite, show a viremia, their health otherwise being unaffected (Hammon and Reeves 1946) While virus has been isolated from several species of bird mites, it has not been possible to show transmission of the virus by their bite (Reeves et al, 1955, Sulkin et al, 1955, Chamberlain and Sikes, 1955) Hammon and Reeves, 1945, indicate that the vector may not be identical in all epidemic areas. Diverse mosquitoes, *Aedes, Anopheles* and *Culiseta*, have been found naturally infected or could be infected experimentally by feeding (Ferguson, 1954), and a wide range of wild and domestic bird species have been found with antibody following natural infection For all these reasons, Meyer (1953) considers that the ecology of infections in vertebrates could be exceedingly complicated Syverton and Berry, 1941, succeeded in transmitting the disease to animals by means of experimentally infected wood ticks, *Dermacentor andersoni*, ticks so infected could, in turn, convey, through transovarian passage, the virus to their progeny, however, there has been no epidemiologic evidence thus far that the tick is an important vector. The virus has been recovered from a cone-nosed bug, *Triatoma sanguisuga* (Kitselman and Grundman, 1940) caught in nature, however, this bug does not occur in most endemic areas. To conclude, the present concept of the spread of this infection and of St. Louis encephalitis is: An aviophilic mosquito conveys the infection from birds to birds as an inapparent infection, thus maintaining the virus in nature; and from birds to other vertebrates including man and horse, both of which are incidental hosts but can exhibit symptoms of disease (Hammon, 1948, Reeves, 1951) The overwintering reservoir of infection is unknown

The fact that man can be infected with WEE virus without having encephalitis is borne out by antibody surveys in areas where, as far as is known, serologic cross-reactions do not constitute a serious diagnostic problem. Some selected references are: Eklund (1946), who studied 173 sera during an epidemic in Minnesota deriving from persons giving no history of encephalitis and found 3, or 1 7 per cent, positive Hammon and Reeves (1947) in an endemic area of Washington, of 151 sera from persons without a history of encephalitis, found that 12, or 8 per cent, were positive. The findings of La Veck et al (1955) should be described in detail 614 sera were collected from persons in Weld County, Colorado, where human and equine cases arose from 1937 through 1949, and the virus was isolated repeatedly from birds and mosquitoes in the 5 years prior to the survey. Of the sera, 67, or about 11 per cent, had NT antibody, later it was found that a positive reactor had had encephalitis 15 years earlier, but none of the other 613 persons had a history of CNS illness. Hence, one can conclude that about 11 per cent of this population in an endemic area responded to infection without clinical manifestations (inapparent infection)

Control Measures

Control measures can be directed either toward preventing dissemination of the virus through an attack on the mosquito vector or toward increasing the resistance of the exposed group through immunizing procedures The former method has been advocated and used extensively in certain endemic areas

immunity. Vaccination of guinea pigs to a degree at which they resist 1,000 cerebral lethal doses fails to protect the CNS against the initial effects of the virus given intracerebrally, an abortive infection of 20 to 30 hours' duration ensues, characterized by fever and histopathologic changes simulating those which occur in control animals at a very early period after infection. In addition, recovered guinea pigs or mice are resistant for about 2 weeks to certain heterologous agents, such as eastern equine and vesicular stomatitis viruses, given intracerebrally. This appears to be an interference phenomenon, Schlesinger et al, 1944 Schlesinger, 1949, has found that vaccinated mice may be resistant to large intracerebral inocula of a slowly growing strain of the virus but not to a rapidly growing derivative Also, if their immunity is of a low grade, they are more resistant to a large than to a small amount of virus, a result, perhaps, of the antigenic booster effect of the excess of virus in the large inocula. Fastier (1952) suggested that the virus given intravenously in young (10 to 15 Gm) mice exhibits toxicity which can be neutralized by specific antiserum

Diagnosis

As with other arbor viruses, the type of disease can only be determined by isolation and identification of the virus or by serologic and immunologic tests. the laboratory procedures involved in diagnosis have been described (cf Chap 10) For general diagnostic use, the CF test is advised, with it, overlapping of WEE antibodes with other Group A viruses is not marked In addition, the slower rate of development of CF as compared with NT or HI antibodies more often permits the demonstration of conversion from negative to positive reaction with paired sera from patients Lennette et al. (1956) called attention to the value of a chick-embryo-derived antigen for CF tests For attempts to isolate virus, the most satisfactory source of virus is CNS tissue; even though this virus has been recovered from the blood and the cerebrospinal fluid of patients, it has been found so rarely as to make viral studies on such material inadvisable as a diagnostic measure.

Treatment

There is no specific treatment of the disease, supportive measures are indicated and essential, and good nursing care is of the utmost importance (Longshore and Maranda, 1956). Olitsky et al., 1943, have shown in experiments with mice and guinea pigs that hyperimmune rabbit serum is ineffective if treatment is begun after onset of definite signs of encephalitis; however, if the antiserum is given to guinea pigs 24 to 48 hours after inoculation of virus and before the signs of disease are obvious, treatment can prevent lethal infection (For experiments with hamsters, see de Boer et al, 1955.) Moreover, in certain serum-treated animals there occurred typical, fatal encephalitis after an incubation period of 13 to 47 days, in such instances, it was assumed that the virus persisted within the CNS during the prolonged incubation period, and when the content of antibody of the CNS reached a low level, even though still demonstrable, it became ineffective and could no longer prevent the active agent from pass- [illegible] fecting other cells These findings

Rivers, 1939, that treatment with immune sera is, with few exceptions, valueless if it is begun after onset of definite clinical signs of a viral infection Sulfonamide compounds and ACTH do not affect the virus or the experimental disease induced by it (Olitsky and Saenz, 1948)

Epidemiology

The main epidemiologic features of the disease are similar to those of St Louis encephalitis, indeed, mixed epidemics have been reported Epidemics of the disease and poliomyelitis have occurred simultaneously in the same area as was the case in Manitoba during the summer of 1941, when, without serologic tests, it was not easy to differentiate one infection from the other, similar mixed epidemics have occurred in western areas of the United States The disease prevails chiefly from early June to mid-September in the United States west of the Mississippi River; equine cases have been reported, in addition, [illegible] and Alabama, and the virus has

1,000 cells among which polymorphonuclear cells predominated during early stages and mononuclear cells later Protein was increased, *but sugar was normal in amount.*

The severity of the disease when clinically

the majority of survivors had sequelae of variable severity However, there are a certain number of human beings who, as first shown by Olitsky and Morgan, *1939*, develop no clinical reaction after infection with the virus but show specific NT antibody (see below).

Pathologic Picture

On macroscopic examination of the 1938 Massachusetts cases, generalized visceral congestion and pulmonary edema were observed The brain showed marked congestion, edema and flattening of the convolutions. On microscopic examination, inflammation of the meninges was noted, and lesions were found to be widespread throughout the brain but mainly in the brain stem and basal ganglia, the cord often being spared or exhibiting only mild changes The pathologic changes in the nervous tissue consisted of severe destruction of neurons and ground substance, perivascular cuffing, and plaques of encephalomalacia In acute cases, the infiltrating cells were polymorphonuclear leukocytes, in patients who succumbed a week after onset of illness, mononuclear cells predominated Small blood vessels revealed disorganization of their walls and endarteritis with deposition of fibrin and formation of thrombi

Experimental Infection, Host Range

The EEE virus is, in general, more invasive and has a greater degree of virulence in experimental animals than has the virus of WEE, this is reflected by a shorter incubation period, more rapid death and often a higher titer of virus in the CNS tissues For example, adult mice are killed within 2 or 3 days after intracerebral exposure to the virus. Otherwise, it induces an experimental disease similar to that caused by the western virus, and the host range is also similar with the exception that cats, hedgehogs, quail and sheep have been shown to be susceptible to the virus given intracerebrally (see above, western equine encephalitis). Of special interest for its epidemiologic implications is the susceptibility to this virus exhibited by a

inoculation or by bite of infected mosquitoes Some of the birds, among which were the white ibis and the purple grackle, developed only subclinical infections with viremia; others, like cardinals and sparrows, developed a fulminating infection with death within 48 hours

Etiology

The properties of EEE virus are, in general, similar to those of WEE virus (see above) Studies by Beard, 1945, and Sharp (*1953*) showed that the infective component of this virus, purified by ultracentrifugation, has a sedimentation constant of 273 S instead of 265, which was found for WEE virus It was also reported by Beard, 1945, that the number of particles of EEE virus per 0 05 cc necessary for infection of mice is 250 However, it should be borne in mind that the validity of such figures necessarily depends on the purity of the preparation being studied (see above, WEE) Bang and Gey, *1949*, found by electron microscopy diplococcuslike bodies in chick-embryo cultures of the virus, similar to those described by Beard in purified preparations of the same agent. The same authors (1952) noted in roller-tube cultures of rat cells deriving from different types of mesenchymatous tissue varying susceptibilities to the virus, some showing slight, others complete destruction, cells of intermediate susceptibility supported virus growth after a single inoculation for over 4 months and developed such minimal changes as not to exceed those seen in uninfected cells This is significant in view of the long persistence of certain viruses in some hosts apparently otherwise unaffected TenBroeck and Herriot reported, 1946, that the active agent is inactivated by one of the mustard compounds, bis(beta-chloroethyl) sulfide, without losing its antigenicity. It could be maintained in 5 per cent guinea pig serum plus 0 1 per cent cysteine

(*Reeves, 1951, Reeves et al, 1952; Stead and* Peters, 1953) A formolized vaccine made from nervous tissue of animals was prepared by Shahan and Giltner, 1934, and Cox and Olitsky, 1936. A formolized chick-embryo vaccine is available for the prevention of the disease in equine animals, Beard et al, 1940 It has received the approval of the U S Bureau of Animal Industry as an effective means of prophylaxis. Recommendations concerning the use of such a vaccine in man await the results of controlled tests in the field under epidemic conditions In the meantime, it is being used for workers in laboratories or those especially exposed to the virus. Specific antiserum for passive immunization has been used successfully in experimental animals, but no satisfactory trial has been made in human beings Studies by Morgan, 1941, and Schlesinger et al (1942) show that prevention of peripheral infection of mice could be achieved when small amounts of serum antibody were present (cf de Boer et al, 1955) Sabin (1951) reported that passive (intravenous) transfer of antibody sufficient to yield an intracerebral neutralization index of ca 50, protected mice against 400 to 4,000 intraperitoneal doses (equal to 2 million mouse intracerebral LD_{50}) of virus Hence, the minimal dose of antibody that might perhaps be considered for trial in human beings should be enough just to show itself in undiluted serum at the end of the period of the individual's exposure—a situation applying, perhaps, to other arbor viruses

EASTERN EQUINE ENCEPHALITIS

(SYNONYMS Similar to those for western equine encephalitis, EEE)

INTRODUCTION

Eastern equine encephalitis is a summer disease of equine and avian animals, which is transmissible to man in whom it can produce extensive inflammation and destruction of CNS tissue

HISTORY

During the summer of 1933, TenBroeck and Merrill recognized an encephalitis in horses on farms in Virginia, Delaware, New Jersey and Maryland. These workers and, at about the same time, Giltner and Shahan, 1933, isolated a new virus from the brains of affected animals, which was designated eastern equine encephalitis virus It was first recovered from human CNS tissue by Fothergill et al, 1938, and Webster and Wright, 1938, from fatal cases of an epidemic that occurred that year in Massachusetts In this epidemic, the first on record, at least 34 persons, mostly children, were involved with a mortality rate of 74 per cent. In the same area and at the same time, 90 per cent of 248 horses which had encephalitis died Subsequently, small but usually severe outbreaks have occurred in man in different localities. Thus, in Louisiana in 1947, at least 15 cases with 9 deaths were reported at the same time that an epizootic occurred in horses of which 90 per cent of 14,000 died In the Dominican Republic in 1948-49, 9 deaths occurred among 13 persons affected, also shortly after an equine outbreak had begun during which 516 cases in horses were reported (Eklund et al., 1951) Additional, sporadic cases, or smaller outbreaks, have been reported in Texas (1941-42) and Massachusetts (1955)

CLINICAL PICTURE

In general, the clinical picture has been similar to the one first described for the 1938 Massachusetts epidemic by Farber et al, 1940, and Ayres and Feemster, 1949 On that occasion, the disease was severe and fulminating As a rule, there were two phases The first, or systemic phase, began suddenly with nausea, vomiting, headache and fever which lasted 24 to 36 hours and was followed by a short period of well-being After this came the second, or encephalitic phase, with high fever reaching 106° F at times, gastro-intestinal disturbances, drowsiness or coma, convulsions, generalized rigidity or opisthotonos, paralyses, bulging fontanelles, edema of legs and face, and cyanosis As a rule, the acute manifestations lasted about a week, extremes being from 1 day to 3 weeks Of the survivors, only 1 recovered completely; the others exhibited sequelae varying from emotional instability to various types of paralyses and mental deterioration

Leukocytosis of 14,000 to 66,000 cells, of which 90 per cent were polymorphonuclear, was present The cerebrospinal fluid was under increased pressure with a pleocytosis up to

1,000 cells among which polymorphonuclear cells predominated during early stages and mononuclear cells later. Protein was increased, but sugar was normal in amount

The severity of the disease when clinically diagnosable as encephalitis is indicated by the fact that in 4 outbreaks (2 in Massachusetts, and 1 each in Louisiana and Dominican Republic) of 66 persons affected, 44 died; and the majority of survivors had sequelae of variable severity However, there are a certain number of human beings who, as first shown by Olitsky and Morgan, 1939, develop no clinical reaction after infection with the virus but show specific NT antibody (see below).

Pathologic Picture

On macroscopic examination of the 1938 Massachusetts cases, generalized visceral congestion and pulmonary edema were observed The brain showed marked congestion, edema and flattening of the convolutions On microscopic examination, inflammation of the meninges was noted, and lesions were found to be widespread throughout the brain but mainly in the brain stem and basal ganglia, the cord often being spared or exhibiting only mild changes The pathologic changes in the nervous tissue consisted of severe destruction of neurons and ground substance, perivascular cuffing, and plaques of encephalomalacia In acute cases, the infiltrating cells were polymorphonuclear leukocytes, in patients who succumbed a week after onset of illness, mononuclear cells predominated Small blood vessels revealed disorganization of their walls and endarteritis with deposition of fibrin and formation of thrombi

Experimental Infection, Host Range

The EEE virus is, in general, more invasive and has a greater degree of virulence in experimental animals than has the virus of WEE, this is reflected by a shorter incubation period, more rapid death and often a higher titer of virus in the CNS tissues For example, adult mice are killed within 2 or 3 days after intracerebral exposure to the virus Otherwise, it induces an experimental disease similar to that caused by the western virus, and the host range is also similar with the exception that cats, hedgehogs, quail and sheep have been shown to be susceptible to the virus given intracerebrally (see above, western equine encephalitis) Of special interest for its epidemiologic implications is the susceptibility to this virus exhibited by a number of domestic and wild birds, pheasants have been shown to be highly reactive to subcutaneous or oral exposure to the virus. Kissling et al. (1954) found 8 species of wild birds susceptible to the virus either by subcutaneous inoculation or by bite of infected mosquitoes Some of the birds, among which were the white ibis and the purple grackle, developed only subclinical infections with viremia; others, like cardinals and sparrows, developed a fulminating infection with death within 48 hours

Etiology

The properties of EEE virus are, in general, similar to those of WEE virus (see above) Studies by Beard, 1945, and Sharp (1953) showed that the infective component of this virus, purified by ultracentrifugation, has a sedimentation constant of 273 S instead of 265, which was found for WEE virus It was also reported by Beard, 1945, that the number of particles of EEE virus per 0 05 cc. necessary for infection of mice is 250 However, it should be borne in mind that the validity of such figures necessarily depends on the purity of the preparation being studied (see above, WEE). Bang and Gey, 1949, found by electron microscopy diplococcuslike bodies in chick-embryo cultures of the virus, similar to those described by Beard in purified preparations of the same agent. The same authors (1952) noted in roller-tube cultures of rat cells deriving from different types of mesenchymatous tissue varying susceptibilities to the virus, some showing slight, others complete destruction; cells of intermediate susceptibility supported virus growth after a single inoculation for over 4 months and developed such minimal changes as not to exceed those seen in uninfected cells This is significant in view of the long persistence of certain viruses in some hosts apparently otherwise unaffected. TenBroeck and Herriot reported, 1946, that the active agent is inactivated by one of the mustard compounds, bis(beta-chloroethyl) sulfide, without losing its antigenicity. It could be maintained in 5 per cent guinea pig serum plus 0 1 per cent cysteine

hydrochloride for at least 11 years at 4° C. (Labzoffsky et al., 1955).

Morgan, 1941, found that following extraneural injection of EEE virus to young and old mice, the capacity to be immunized increased with the age of the animal, i.e., old mice produced a higher level of antibody at a more rapid rate than did young ones With EEE and WEE viruses, Olitsky et al., 1936, and Sabin and Olitsky, 1939, demonstrated in mice and guinea pigs the development with age of resistance to the effects of peripheral inoculation

Diagnosis

Specific diagnosis of the infection can be made only by laboratory procedures The virus has been isolated from the blood of birds naturally infected and of sentinel monkeys. All recorded isolations of the virus from human beings are from CNS tissue, it is conceivable that blood taken at the time of viremia, preceding CNS involvement, might yield the agent.

Treatment

No specific treatment is available Sulfonamide and antibiotic compounds are ineffective in experimental infections

Epidemiology

The disease in horses and mules is, at present, distributed over the eastern part of the United States and Canada (Ontario), Mexico, Brazil, Panama, Cuba and the Dominican Republic In pheasants, the infection is prevalent in the northeastern states, particularly New Jersey where, between 1939 and 1954, 27 epizootics have been reported (Beaudette et al, 1955) The virus has been isolated from a monkey in the Philippine Islands In man, clinical infection with EEE virus has been reported in Massachusetts, Texas and Louisiana and in Cuba and the Dominican Republic Human outbreaks usually have been preceded or accompanied by epizootics in horses, this was the case in Massachusetts in 1938, Louisiana in 1947, and in the Dominican Republic in 1948-49 In Massachusetts, during July through October, 1938, the disease attacked human beings and horses, the median date for reported deaths in horses was August 27, 2 weeks earlier than a similar date for human deaths The majority of cases in this epidemic occurred in young children and infants, 70 per cent were under 10 years, 25 per cent less than 1 year; both sexes were attacked equally. During the fall, the disease was detected in pheasants and a pigeon caught in the same area.

The severity of the clinical disease in the United States has been mentioned already (see above), it is also worthy of note that evidence of subclinical infection with this virus in northeastern United States has not been reported, in general, Fothergill (1939) failed to detect neutralizing antibodies in the serum of 114 persons, many of whom were contacts. Similarly, Liao (1955) found no antibodies in the serum of 215 pheasant farmers and handlers after a severe epizootic in pheasants in Connecticut However, neutralizing antibodies have been detected in other localities in the absence of encephalitis, indicating that subclinical or aberrant infections occur; surveys carried out shortly after epidemic outbreaks showed neutralizing antibody in Louisiana in 4 of 45 persons (Howitt et al., 1948); in the Dominican Republic in 32 of 827 (Eklund et al, 1951). In addition, antibodies have been detected in instances where no association with an outbreak was apparent, as reported by Schaeffer et al (1954), in Louisiana with 4 positive of 69 tested, and by Causey and Theiler (1958) in Brazil with 16 positives among 326 persons The fact that in northeastern United States no positive sera were found of 329 tested (cf Fothergill and Liao), while in the Dominican Republic 32 of 827 were positive and in Brazil, 16 of 326, may point to differences in epidemiologic patterns

Merrill et al., 1934, showed that EEE virus multiplies in *Aedes sollicitans* mosquitoes while WEE virus multiplies in *Aedes aegypti*, thus demonstrating for the first time multiplication in mosquitoes of a virus derived from animals. Chamberlain et al (1954) investigated the experimental vector potential for EEE virus of about 20 different species of mosquitoes, on the basis of thresholds of infection, infection rates and transmission rates for each species, certain mosquitoes (*Aedes aegypti*, *A sollicitans* and *A. triseriatus*) are considered more likely to be active vectors than others

The vector in nature is not known The

virus has been isolated from the following wild caught mosquitoes; *Mansonia perturbans*, by Howitt et al. (1948), *Culiseta melanura*,

chicken mites (*Dermanyssus gallinae*) and a mixture of lice (*Eomenacanthus stramineus* and *Menopon pallidum*) It is worthy of note that, in endemic areas in the United States, the frequency of isolations of EEE virus from wild caught arthropods is far lower than that of other arbor viruses, i e, WEE and St Louis, in their endemic areas

Studies on the possible reservoir of the virus in nature, by TenBroeck in 1938 and 1940, led to the conclusion that birds are more likely than horses to act as reservoir hosts Additional experimental evidence pointing in the same direction has been offered by Kissling et al (1951), who found several species of wild birds to be susceptible to this agent, the birds developed viremia without other signs of illness Furthermore, EEE virus has been isolated in nature from the blood of birds on four occasions purple grackle (Kissling et al., 1954), loggerhead shrike, cardinal and Carolina chickadee (Kissling et al, 1955) The observations by Kissling et al (1954) that antibody that developed in birds after naturally acquired infection was transmitted to nestlings via the ovary, and a similar observation by Reeves et al (1954) for WEE and St Louis viruses might indicate a possible mechanism for limiting the natural spread of infection among nestling birds

The present estimate of the situation prevailing with EEE virus infection concerning reservoir-vector-host relationship is summarized by Schaeffer and Arnold (1954) in their review of the result of a 5-year study Equine animals are probably a "dead end" in the infection chain, and transmission to human beings is only occasional and incidental Birds and mosquitoes remain important in the natural history of the infection, while the role of mites or other ectoparasites is considered of minor or probably no importance.

A particularly interesting aspect concerning the dissemination of this virus in nature is presented by outbreaks in pheasants From the mode of spread of the disease which affects nearly all the birds in one pen while failing to affect birds in adjacent pens, in addition to the fact (Holden, 1955b) that pheasants can be infected by ingestion of the virus, it would appear that the epidemiologic cycle in the pheasant can be completed without an insect vector.

Control Measures

Specific antiserum prevents the disease in experimental animals, its use as a prophylactic measure in man must await further tests Formalin-inactivated vaccines prepared from infected chick embryos, Beard et al, 1940, have been used successfully for prevention of the disease in equine animals The general principles of active immunization with such vaccines were described by Olitsky and Cox, 1936, and by Cox and Olitsky, 1936 While the vaccine has not as yet had a trial in human beings in the face of an epidemic, it has been given to persons who from the nature of their work are exposed to the virus, as yet it is not recommended for mass immunization of human populations Hurst et al (1952) stated that the antimalarial mepacrine (atabrine) inhibits multiplication of the virus and is, perhaps, a preventive of the experimental disease in mice, however, after definite lesions have developed, it is ineffective Mosquito control should be considered in any program for prevention of the disease

VENEZUELAN EQUINE ENCEPHALITIS

(Synonyms Peste loca; VEE)

Introduction

Venezuelan equine encephalitis is observed as a disease primarily of equine animals, its causal agent is transmissible to human beings in whom it usually induces a mild disease of variable syndrome, rarely, if ever, associated with frank encephalitis

History

During 1935, an encephalitis was observed in horses and mules in Colombia In 1938, a severe epizootic swept over Venezuela, the agent responsible for this outbreak was recovered by Beck and Wyckoff, 1938, and by Kubes and Rios, 1939, from the brains of animals that had died of the disease Later, the virus was reported in Ecuador, Trinidad,

Panama and more recently (Causey and Theiler, 1958) in Brazil. Human infections have been described by Casals et al, 1943, and by Lennette and Koprowski, 1943, they occurred in laboratory workers and were mild, other laboratory infections have been reported in Argentina in 1944. Two fatal encephalitic infections of man, possibly due to VEE virus, occurred in 1943 in Trinidad and were described by Randall and Mills, 1944, and Gilyard, 1945 In the Colombian epidemic of 1952, at first the cases were diagnosed as a denguelike fever (Sanmartin-Barberi et al, 1954)

Clinical Picture

The precise incubation period is unknown; it is probably short, from 2 to 5 days As a rule, persons infected with this virus during laboratory investigations do not show neurologic or encephalitic signs but a picture similar to influenza, generally with mild symptoms, headache and fever are prominent, also gastrointestinal disturbances. Only in rare instances are tremor, myalgia, diplopia and lethargy noted Signs and symptoms persist for 3 to 5 days in mild cases and for 8 days in more severe attacks, after which prompt and complete recovery takes place In the 2 cases in Trinidad, acquired under natural conditions, the onset was acute with definite symptoms of encephalitis which were followed by coma and death In the epidemic in Colombia in 1952, *perhaps as many as 70 cases occurred*, the clinical picture as subsequently reconstructed was marked by sudden onset with malaise, chills and fever, nausea, vomiting, headache, muscle and bone pains Fever lasted from 1 to 4 days, and complete recovery ensued Little is known as yet concerning changes in the blood and the spinal fluid.

The pathologic picture in human beings has not been sufficiently studied to warrant comment, while in the experimental disease of guinea pigs, rabbits and mice, a lymphomyelopoietic necrosis is seen and encephalomyelitis is noted in monkeys and mice The necrosis involves all lymphoid and myeloid tissues, and the CNS lesions are not much different from the general picture in animals produced by other arbor viruses (Victor et al, 1956). The horse, which usually shows pancreatic lesions, is susceptible to intranasal instillation of the virus; infected animals shed virus from the nose, the eyes, oral secretion, urine and milk; they can infect by contact (Kissling et al., 1956).

Experimental Infection; Host Range

In addition to equine animals, the virus is pathogenic by intracerebral route for mice, guinea pigs, rabbits, rats, dogs, cats, sheep, goats, and partially so for pigeons Chick embryos are susceptible. Cattle are resistant A distinctive feature of the virus is its high virulence for adult animals when inoculated by peripheral (non-neural) routes, when administered in this way, as little as 10^{-9} or 10^{-10} dilutions of mouse-adapted virus may infect mice. This species responds to intracerebral introduction of the virus with a disease that is transmissible to normal mice by contact in cages. Such infected mice harbor the virus in the tracheopharyngeal washings and the feces but not in the urine In the natural disease of man, the virus had been found in the CNS and also in the blood. The active agent is recovered not only from the nasopharynx of human beings infected accidentally in the laboratory but also from their blood; this is a characteristic feature of the malady It is important to recognize that this virus actively infects human beings as well as experimental animals exposed to it in laboratories.

Etiology

Little is known of the properties of the virus. It is not readily inactivated by formalin in vaccine preparation (see below). It is filterable through Berkefeld N and Seitz filters; it can be preserved in 50 per cent buffered glycerol by being held in the frozen state at $-70°$ C and by lyophilization It grows well in the Maitland type of tissue culture and in embryonated hens' eggs; active growth is possible in human and canine uterine tissue cultures (Gajdusek et al, 1954) and in HeLa cells (Murphy et al, 1955) Convalescent human beings and animals immunized with vaccines develop specific NT, CF and HI antibodies, they appear from 1 to 2 weeks after onset of infection, but how long they endure is still to be determined

Diagnosis

A specific diagnosis can be made only by laboratory procedures, *i e., by identification*

of the virus isolated from CNS tissue, blood or nasopharyngeal washings, or by finding HI, NT or CF antibodies during convalescence

Treatment

There is no specific treatment

Epidemiology

The known distribution of the natural disease or of the virus is, at present, in northern South America, the Amazon Valley, Trinidad and Panama The reservoir of the virus is still to be found Its mode of transmission in man may vary, in certain instances, such as in laboratory infection, it may be by droplet or dust infection Gilyard, 1944, considers that *Mansonia titillans*, a culicine mosquito, and *Aedes taeniorhynchus* may transmit the disease to horses and human beings Wild birds can be infected by subcutaneous inoculation and by bite of infected *Aedes triseriatus* (Chamberlain et al, 1956), the infection is nearly always inapparent or silent This mosquito can act as a vector in transmission experiments with English sparrows, owing to the low level of viremia, Chamberlain et al (1956) consider the role of birds in this disease uncertain but capable of contributing as a source of infection for the mosquito in the cycle mammal-mosquito Since man may become infected through the upper respiratory tract and since the virus is found in the nasopharyngeal washings of infected human beings, it is conceivable that epidemics may occur without the aid of an insect vector The extent of inapparent infection by this virus is brought out by the studies of Downs et al (1956), which revealed that 6 9 per cent of 160 sera deriving from healthy residents of Trinidad without a history of encephalitis had neutralizing antibodies against VEE virus, and by Causey and Theiler (1958), who found that 16 per cent of 30 similar sera from persons in the Amazon Valley also contained neutralizing antibodies

Control Measures

A formalin-inactivated vaccine prepared from infected chick embryos, Kubes and Rios, 1939, has been used in Venezuela for prevention of disease in horses Randall et al, 1949, prepared a partially purified vaccine which has been shown to produce a high level of antibody. This vaccine has been used only for protection of laboratory workers, Smadel, 1951 A similar vaccine of the 3 types of the equine viruses was prepared later at the United States Army Medical School (Maurer et al, 1952). However, Sutton and Brook (1954) have drawn attention to an important finding which may apply to other viral vaccines. Of those receiving the formalinized Venezuelan virus, 14 of 327 persons (1,174 inoculations) became ill, and 8 of 10 tested yielded this virus Two points are brought out: (1) either the tests for active virus in the vaccine were inadequate, or (2) man is more susceptible to the virus than are the laboratory animals used in the tests Any program for prevention should include insect control

SEMLIKI FOREST VIRUS

(Synonyms: Semliki virus, Kumba virus)

The Semliki Forest virus was first isolated by Smithburn and Haddow, 1944, from a group of *Aedes abnormalis* mosquitoes caught in Uganda To the present time, the virus has been recovered twice from mosquitoes, no human disease caused naturally by it has been reported, nor has the virus been isolated from man

The available strains of virus are pathogenic for mice when introduced into them intracerebrally or peripherally, viremia develops, followed by encephalitis characterized by lesions resembling those of the equine encephalitis viruses Guinea pigs, rabbits, rhesus and red-tail (*Cercopithecus nictitans*) monkeys are susceptible to intracerebral but not to peripheral inoculation of the virus The virus can be propagated in golden hamsters, chick embryos and in tissue cultures (Ginder and Friedewald, 1951)

The virus has been reported to be unusually resistant to heat, and 60° C for 1 hour is needed for its inactivation Its size as determined by gradocol-membrane filtration is 15 to 30 mμ It is cytopathogenic in and destroys the tissue of rabbit fibromata; it is also cytopathogenic in tissue cultures thereof, even though the active agent is nonpathogenic for rabbits by peripheral routes of exposure (Ginder and Friedewald, 1951). The antibiotic helenine derived from *Penicillium funic-*

ulosum was found to modify experimental infection in the mouse (Shope, 1953).

Within Group A, Semliki virus is more closely allied serologically to Chikungunya and Mayaro viruses than to the other agents of the group. Owing to this close serologic relationship, antibodies found in nature either in man or in animals, capable of reacting with Semliki virus, must be interpreted with caution until their specificity is ascertained. Antibodies in man capable of neutralizing Semliki virus have been reported in Uganda (Smithburn et al., 1944), Malaya and Borneo (Smithburn, 1954), India (Smithburn et al., 1954), South Africa (Kokernot et al., 1956) and Brazil (Causey and Theiler, 1958). On the basis of HI tests using an antigen developed by Clarke and Theiler (1955), it would appear as though the antibodies found in Brazil can best be explained by the presence of Mayaro virus in that country (Casals and Whitman, 1957). Limited information indicates that, in some cases, human sera from Africa and Malaya having neutralizing antibodies against Semliki virus react by HI test to a considerably higher titer against Chikungunya than against Semliki virus.

CHIKUNGUNYA DISEASE

Introduction

Chikungunya is a newly described human disease first noted in 1952-1953 in Tanganyika. It takes its native name from its outstanding symptom, i.e., a state of being "doubled-up" owing to fulminating, severe joint pains that compel this striking posture.

History

An outbreak of a denguelike malady arose in the Makonde Plateau, Newala district of southern Tanganyika. A virus was isolated repeatedly by Ross (1956) from patients' blood in the acute phase, also from wild caught mosquitoes. The incitant was then shown to be a new member of Group A arbor viruses (Spence and Thomas, 1958). Recently, additional strains have been recovered from the blood of an African mosquito catcher in the Zika forest near Entebbe, Uganda; and from human cases of denguelike disease in the Union of South Africa (Smithburn, 1957).

Clinical Picture

The following account derives from Robinson's (1955) observations on 115 hospitalized patients and on a number of other affected individuals. The incubation period is 3 to 12 days. The onset is sudden with excruciating pains in the joints, the patients being incapacitated within a few minutes or hours from the onset. A rapid rise of temperature to 102° to 105° F. supervenes, and the victim assumes a doubled-up position and becomes immobile, hence, the name of this illness. The pains occur in the limbs and the spine, but headache is usually mild, and no eyeball or postorbital pains, as found in dengue, are noted, although anorexia and constipation occur. After 1 to 6 days of fever, an apyrexial period of 1 to 3 days is followed in most patients by another less intense fever. At this recurrence, or thereafter, a rash is seen in about 80 per cent of individuals, which is maculopapular, pruritic and present on the trunk and on the extensor surfaces of the limbs. The malady in this acute stage endures 6 to 10 days, but in several persons apyrexial joint pains recur intermittently over a period up to 4 months, and at times they practically cripple the victim. No second attacks have been known to arise, no deaths have been ascribed to the disease. The blood reveals a leukopenia of 4,000 to 5,000 cells. No pathologic reports on patients or experimental animals are as yet available.

Experimental Infection; Host Range

The virus is pathogenic for suckling mice after intracerebral or intraperitoneal inoculation. The LD_{50} titer by the former route is 10^{-7}, and the incubation period 2 to 5 days. Adult (28-day-old) mice were not as highly susceptible (Ross, 1956) even when the suckling-mouse-adapted strain in its 100th passage was given, but with the 160th passage they developed a lethal infection with only a few survivors; the titer was 10^{-6}, and the incubation period 4 to 8 days; they succumbed after 3 to 5 days. Guinea pigs and rabbits are insusceptible.

Etiology

The properties of the virus have not as yet been sufficiently studied. Immunologically, this virus and Mayaro and the Semliki con-

stitute a subgroup in Group A, since these 3 members are more closely related one with another than to others of this group (Casals, 1957, Spence and Thomas, 1958)

Diagnosis

A specific diagnosis can be made only by virus isolation or serologic tests with the patients' sera Care must be taken in interpreting the results of serologic tests, particularly HI, owing to the close relationships existing in the Chikungunya-Mayaro-Semliki viruses complex The clinical differentiation of this disease from dengue rests on the absence of adenitis in Chikungunya as well as frequent association of a rash appearing with or after the secondary fever, absence or mildness of headache, lack of pains postorbitally or with eye movements and characteristic chronicity of joint pains

Treatment

There is no specific treatment, only symptomatic, and opiates are often indicated for alleviation of the arthralgia

Epidemiology

The virus was first isolated from the blood of man and from mosquitoes, bedbugs collected in a patient's hut may have also contained the virus Ross (1956) showed the association between the virus and the disease in the Tanganyika outbreak when in 15 paired sera from patients the samples collected between 5 hours and 4 days after onset had neutralization indices between 0 3 or less and 1 1, but in convalescence, 8 to 16 days after onset, the indices were between 2 1 and 2 3 or higher Spence and Thomas (1958) by HI test demonstrated antibodies in 16 per cent of 25 acute-phase sera, while in a group of 40 convalescent-phase sera, 72 per cent were positive

The epidemic extended from July, 1952, to March, 1953, the majority of cases arose between November and February with most of them seen in January The total number of persons attacked is not known, however, of 2,093 persons living in a number of localities, 860 came down Native Africans, Asians and Europeans were equally involved Persons over 45 years old seemed in some areas to be resistant When the illness attacked one, all the other inmates of a hut developed it; within 2 or 3 weeks, 60 to 80 per cent of a village became sick

In Africa, the clinical disease is believed to be present also in Portuguese East Africa The virus has been isolated from man in Tanganyika, Uganda and Union of South Africa Limited information by means of HI tests indicates that infection with Chikungunya virus may cause either subclinical or milder manifestations than described above, and that this virus, or one very closely allied to it, may be active in other localities Thus (Casals and Theiler, 1956) in the absence of any recorded disease resembling the one in Tanganyika, antibodies against this virus were found in the following areas 11 of 22 residents in Malaya, 10 of 42 in the Union of South Africa, and 35 of 64 in Nigeria

The virus was recovered from two species of mosquitoes trapped in huts *Culex fatigans* and *Aedes aegypti* An additional strain was secured recently from *Aedes africanus* caught in the Zika forest Lumsden (1955) considers *Aedes aegypti* as the vector on epidemiologic grounds Ross (1956) states that the virus could be recovered from mosquitoes fed on patients during their acute illness as late as 33 days after the infected meal Whitman (1956) finds the virus capable of multiplying in the body of *Aedes aegypti* by salivary-gland tissue transfer in series in mosquitoes, since each transfer represents a dilution of 10^{-5} by weight of gland, such a prodigious increase of virus would support its classification as arthropod-borne

There is, at present, no means for prevention except mosquito control or abatement

MAYARO VIRUS DISEASE

Five cases of a mild, febrile malady were observed in 1954 in residents of Trinidad, B W I, from each, a hitherto undescribed virus was isolated by intracerebral inoculation of acute-phase serum into newborn mice The virus was named Mayaro after the district where the first case was observed (Anderson et al, 1957) Shortly afterward, in 1955, an epidemic which may have involved about 50 persons, erupted along the Guama River, Para, Brazil, and from 6 patients additional strains of Mayaro virus were isolated (Causey

ulosum was found to modify experimental infection in the mouse (Shope, 1953)

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CHIKUNGUNYA DISEASE

Introduction

Chikungunya is a newly described human disease first noted in 1952-1953 in Tanganyika It takes its native name from its outstanding symptom, *i e*, a state of being "doubled-up" owing to fulminating, severe joint pains that compel this striking posture

History

An outbreak of a denguelike malady arose in the Makonde Plateau, Newala district of southern Tanganyika. A virus was isolated repeatedly by Ross (1956) from patients' blood in the acute phase, also from wild caught mosquitoes The incitant was then shown to be a new member of Group A arbor viruses (Spence and Thomas, 1958). Recently, additional strains have been recovered from the blood of an African mosquito catcher in the Zika forest near Entebbe, Uganda; and from human cases of denguelike disease in the Union of South Africa (Smithburn, 1957)

Clinical Picture

The following account derives from Robinson's (1955) observations on 115 hospitalized patients and on a number of other affected individuals. The incubation period is 3 to 12 days. The onset is sudden with excruciating pains in the joints, the patients being incapacitated within a few minutes or hours from the onset. A rapid rise of temperature to 102° to 105° F. supervenes, and the victim assumes a doubled-up position and becomes immobile, hence, the name of this illness. The pains occur in the limbs and the spine, but headache is usually mild, and no eyeball or postorbital pains, as found in dengue, are noted, although anorexia and constipation occur. After 1 to 6 days of fever, an apyrexial period of 1 to 3 days is followed in most patients by another less intense fever. At this recurrence, or thereafter, a rash is seen in about 80 per cent of individuals, which is maculopapular, pruritic and present on the trunk and on the extensor surfaces of the limbs The malady in this acute stage endures 6 to 10 days, but in several persons apyrexial joint pains recur intermittently over a period up to 4 months, and at times they practically cripple the victim. No second attacks have been known to arise, no deaths have been ascribed to the disease The blood reveals a leukopenia of 4,000 to 5,000 cells No pathologic reports on patients or experimental animals are as yet available

Experimental Infection, Host Range

The virus is pathogenic for suckling mice after intracerebral or intraperitoneal inoculation. The LD_{50} titer by the former route is 10^{-7}, and the incubation period 2 to 5 days. Adult (28-day-old) mice were not as highly susceptible (Ross, 1956) even when the suckling-mouse-adapted strain in its 100th passage was given, but with the 160th passage they developed a lethal infection with only a few survivors, the titer was 10^{-6}, and the incubation period 4 to 8 days, they succumbed after 3 to 5 days. Guinea pigs and rabbits are insusceptible.

Etiology

The properties of the virus have not as yet been sufficiently studied Immunologically, this virus and Mayaro and the Semliki con-

delta, of over 400 persons whose sera were tested, 27 per cent had antibodies, in those under 15 years, the percentage of positives was 14, and in those older than 15 years, 34 In the Union of South Africa (Weinbren et al, 1956), 9 per cent of 160 non-European residents had antibody against the virus

MIDDELBURG VIRUS

From wild caught mosquitoes, *Aedes caballus* and *Aedes* (*Banksinella*) sp, Kokernot et al (1957), isolated at least two strains of a hitherto undescribed agent which was named Middelburg virus from the district in the eastern Cape Province, South Africa, where the agent was recovered They showed that the virus belongs in Group A and is transmissible experimentally by *Aedes caballus* In addition, newborn mice were found to be susceptible to intraperitoneal as well as intracerebral inoculation, adult mice were insusceptible Lambs developed a rapid febrile response (to 106° F) following inoculation of the virus, no other signs of illness were observed, and recovery was the rule In a limited survey, neutralizing antibodies were found in 2 of 9 persons and 10 of 13 ewes Although an epizootic was present in sheep at the time of the isolation of this agent, its connection with the outbreak in sheep was not established

GENERAL COMMENTS ON GROUP A, ARBOR VIRUSES

Certain practical usages and theoretical considerations regarding the classification here presented are in order

On the basis of serologic reactions, including NT, HI and CF tests, with experimental animal sera, it is possible to show that in Group A there exist 2 subgroups or complexes characterized by the fact that the viruses in each subgroup are more closely related to one another than to the remaining agents in Group A These subgroups are (1) Chikungunya, Mayaro and Semliki Forest viruses and (2) WEE and Sindbis

The serologic overlaps shown in the Chikungunya-Mayaro-Semliki complex are apt to be quite marked and can present difficult diagnostic problems, mention has been made earlier in this chapter of the revised interpretation placed on the detection of neutralizing antibody against Semliki Forest virus in the Amazon Valley In such instances, it is advisable to test the sera under investigation against all 3 agents; or, if the diagnostic problem involves a virus isolate, to test the virus against the 3 immune sera; the nature or pattern of the serologic results is of definite help. Further, the study of the characteristics of the virus, of the clinical, ecologic and epidemiologic pictures can be of additional aid in resolving overlapping reactions

The WEE-Sindbis relationship, as observed with experimental animal sera by HI and CF tests, is characterized by a strong overlap given by Sindbis serum against WEE antigen, the converse reaction, on the other hand, is usually more moderate.

The equine viruses (WEE, EEE, VEE) are agents which, serologically, are different from each other But one of the essential points of similarity among the 3 is that ordinarily the encephalitis in man is unusual or accidental, the usual form of infection being, at least with WEE and VEE (less is known about EEE), asymptomatic, abortive, or systemic Indeed, VEE is almost always a systemic disorder, often resembling the clinical picture of influenza or dengue, although the horse may show encephalitis For WEE and EEE, the order of susceptibility is probably first woodland animals (especially birds), then horses and last human beings

Finally, antibody surveys for Group A arbor viruses, made under conditions favorable for proper interpretations despite cross-reactions, reveal that large numbers of the general population in endemic and epidemic areas develop clinically inapparent infections Even with the virus of EEE with which, in the earlier surveys, there was little evidence of subclinical infection, there seem to be an increasing number of reports of persons that have antibody in the absence of recognizable encephalitis

REFERENCES

(For complete references, see also second edition, chapter 11)

Anderson, C R, Downs, W G, Wattley, G H, Ahin, N W, and Reese, A A, 1957, Mayaro virus a new human disease agent II, Isolation from blood of patients in Trinidad, B W I, Am J Trop Med 6, 1012-1016

and Maroja, 1957) A strain of virus identical with or closely related to Mayaro was isolated from man in Bolivia (Buescher, 1955) and was called Uruma virus Antibodies against Mayaro virus have been detected in residents of Colombia (Sanmartin-Barberi, 1956).

The disease as seen in the two areas mentioned was characterized by moderate fever, 100° to 103.5° F, severe frontal headache, occasionally by epigastric pain, backache, nausea, vertigo and mild jaundice; the symptoms lasted from 2 to 6 days

The available strains of the virus are pathogenic for newborn mice, with LD_{50} at about 10^{-7} after intracerebral or intraperitoneal exposure; adult mice do not show visible signs of infection After intracerebral inoculation of some strains of this virus guinea pigs occasionally respond with neurologic signs followed by death

Casals and Whitman (1957) showed by serologic tests that Mayaro virus, in addition to being an arbor Group A agent, is closely related to Semliki Forest virus Further, both Mayaro and Semliki viruses are serologically more closely related to Chikungunya virus than to the remaining agents in Group A, as a consequence (Casals, 1957), the three agents are considered as a serologic subgroup or complex within Group A These close serologic relationships must be borne in mind when interpreting the results of immunologic reactions thus, Causey and Maroja (1957) express the view that neutralizing antibodies against Semliki virus found in 9 6 per cent of 551 persons in the Amazon Valley may be due to infection with Mayaro rather than Semliki virus

SINDBIS VIRUS

Sindbis virus is so named from the Sindbis district in the Nile delta where it was first isolated in 1952 by Taylor (Taylor et al, 1955); several isolations followed from culicine mosquitoes and one from the blood of a hooded crow. The virus was recovered in India in 1953 (Shah et al, 1957) from mosquitoes, mites and birds Early in 1954, an epizootic broke out in cattle, accompanied by an epidemic of a febrile disorder arising from a cause still unknown near Johannesburg, Union of South Africa. From the mosquitoes of the neighborhood, three strains of virus identical with or closely related to the Egyptian Sindbis virus were recovered (Weinbren et al, 1956) The effects of the virus on man are unknown, since as yet no disease has been associated with this agent, nor has the virus been isolated from man

On isolation, the strains of virus available were pathogenic for newborn mice, not for adults Most strains have remained thus; but at least one strain has been adapted to adult mice by serial intracerebral passage (Weinbren et al, 1956) In 1- or 2-day-old mice, the virus produces paralysis and death in 2 to 6 days after intracerebral or peripheral inoculation, with LD_{50} at about 10^{-7} by the former route and slightly lower by the latter Rabbits react to intracerebral inoculation of the virus with no apparent ill effects In birds, including chicks, and certain monkeys, exposure to the virus sets up a viremia only, without apparent illness. cavebats and hamsters come down with signs after inoculation by several routes (Reagan et al, 1956). The histopathologic picture in affected mice reveals foci of separation, fragmentation and loss of striation of myofibrils along with nuclear degeneration—a Coxsackie viruslike lesion. In the CNS, however, the encephalitis is of a type *sui generis* and shows chiefly neuronal degeneration and necrosis without edema or perivascular infiltration The virus is highly pathogenic for chick embryos which die within 72 hours; in chick-embryo tissue cultures occurs a marked cytopathogenesis of the fibroblastic outgrowths (Frothingham, 1955) By gradocol-membrane filtration, the virus has a particle size between 40 and 48 mμ (Taylor et al, 1955). Within Group A, Sindbis virus has been found to be more closely related serologically with WEE than with the remaining viruses in the group (Casals, 1957).

Experimentally, the virus was easily maintained in several species of *Culex* mosquitoes and transmitted by bite to newborn mice; a tick, *Ornithodoros savignyi*, could be infected by parenteral puncture and was capable, subsequently, of transmitting the infection by feeding upon infant mice.

Antibody surveys by means of NT test suggest a wide vertebrate host range including man, domestic quadrupeds, birds and domestic fowl (Taylor et al, 1955) In the Nile

1951, Western equine and St Louis encephalitis viruses in the blood of experimentally infected wild birds and epidemiological implications of findings, J Immunol 67, 357-367

Holden, P, 1955a, Recovery of western equine encephalomyelitis virus from naturally infected English sparrows of New Jersey, 1953, Proc Soc Exper Biol & Med 88, 490-492

———, 1955b, Transmission of eastern equine encephalomyelitis in ring-necked pheasants, Proc Soc Exper Biol & Med 88, 607-610

Howitt, B F, Bishop, L K, Gorrie, R H, Kissling, R E, Houser, G H, and Trueting, W L, 1949 An outbreak of equine encephalomyelitis, eastern type, in southwestern Louisiana, Proc Soc Exper Biol & Med 68, 70-72

Huang, C H, 1942, Titration and neutralization of the western strain of equine encephalomyelitis virus in tissue culture Proc Soc Exper Biol & Med 51, 396-398

———, 1943, Further studies on the titration and neutralization of the western strain of equine encephalomyelitis virus in tissue culture, J Exper Med 78, 111-126

Hurst, E W, Melvin, P, and Peters, J M, 1952, Prevention of encephalitis due to the viruses of eastern equine encephalomyelitis and louping-ill, experiments with trypan red, mepacrine and many other substances, Brit J Pharmacol 7, 455-472

Karstad, L H, Fletcher, O K, Spalatin, J, Roberts, R, and Hanson, R P, 1957, Eastern equine encephalomyelitis virus isolated from three species of diptera from Georgia, Science 125, 395-396

Kissling, R E, Chamberlain, R W, Nelson, D B, and Stamm, D D, 1955, Studies on the North American arthropod borne encephalitides VIII Equine encephalitis studies in Louisiana Am J Hyg 62, 233-254

———, 1956, Venezuelan equine encephalomyelitis in horses, Am J Hyg 63, 274-287

Kissling, R E, Chamberlain, R W, Sikes R K, and Eidson, M E 1954, Studies on the North American arthropod borne encephalitides III Eastern equine encephalitis in wild birds Am J Hyg 60, 251-265

Kissling, R E, Eidson, M E, and Stamm, D D, 1954, Transfer of maternal neutralizing antibodies against eastern equine encephalomyelitis virus in birds, J Infect Dis 95, 179-181

Kissling, R E, Rubin, H, Chamberlain, R W, and Eidson, M E, 1951, Recovery of virus of eastern equine encephalomyelitis from blood of a purple grackle, Proc Soc Exper Biol & Med 77, 398-399

Kitselman, C H, and Grundmann, A W, 1940, Equine encephalomyelitis virus isolated from naturally infected Triatoma Sanguisuga Le Conte Tech Bull Kansas Agric Exp Sta 50, 15

Kokernot, R H, de Meillon, B, Paterson, H E, Heymann, C S, and Smithburn, K C, 1957, Middelburg Virus A hitherto unknown agent isolated from Aedes mosquitoes during an epizootic in sheep in the eastern Cape Province South African J M Sc 22, 145-153

Kokernot, R H, Smithburn, K C, and Weinbren, M P, 1956, Neutralizing antibodies to arthropod-borne viruses in human beings and animals in the Union of South Africa, J Immunol 77, 313-323

Labzoffsky, N A, Granger, R M, and Ross, W G, 1955, Survival of equine encephalitis virus (eastern type) for 11 years at 4° C in the presence of cysteine hydrochloride, Canad J Microbiol 1, 715-720

La Veck, G D, Winn, J F, and Welch, S F, 1955, Inapparent infection with western equine encephalitis virus epidemiologic observations, Am J Pub Health 45, 1409-1416

Lennette, E H, Wiener, A, Neff, B J, and Hoffman, M N, 1956, A chick-embryo derived complement-fixing antigen for western equine encephalomyelitis, Proc Soc Exper Biol & Med 92, 575-577

Liao, S J, 1955, Eastern equine encephalitis in Connecticut serological survey in pheasant farmers, Yale J Biol & Med 27, 287-296

Longshore, W A, Jr, and Maranda, E J, 1956, Viral encephalitis—current medical and nursing concepts, Am J Nursing 56, 447-450

Longshore, W A, Jr, Stevens, I M, Hollister, A C, Jr, Gittelsohn, A, and Lennette, E H, 1956, Epidemiological observations on acute infectious encephalitis in California, with special reference to the 1952 outbreak, Am J Hyg 63, 69-86

Lumsden, W H R, 1955, An epidemic of virus disease in Southern province, Tanganyika territory in 1952-53 II General description and epidemiology Tr Roy Soc Trop Med & Hyg 49, 33-57

Maurer, E R, Kuttler, K L, Yager, R H, and Warner, A, 1952, Immunization of laboratory workers with purified trivalent equine encephalomyelitis vaccine, J Immunol 68, 109-114

Meyer, K F, 1953, Vectors and reservoirs of virus disease, Am J Trop Med 2, 757-770

Murphy, L C, Hubbell, E J, and Yager, R H, 1955, Propagation of Venezuelan equine encephalomyelitis virus in cultures of human malignant epithelial cells, strain HeLa, Am J Vet Res 16, 304-307

Olitsky, P K, and Saenz, A C, 1948, Serum treatment of western equine encephalitis in mice determined by the course of viral infection, Proc Soc Exper Biol & Med 68, 200-204

Reagan, R L, Yancey, F S, Chang, S C, and Brueckner, A L, 1956, Studies of the Sindbis virus Part I Susceptibility of the cave bat by various routes of exposure Part II Susceptibility of the suckling hamster by various routes of exposure Am J Trop Med 5, 1032-1035

Reeves, W C, 1951, The encephalitis problem in the United States, Am J Pub Health 41, 678-686

Reeves, W C, and Hammon, W McD, 1944, Feeding habits of the proven and possible mosquito vectors of western equine and St Louis encephalitis in the Yakima Valley, Washington, Am J Trop Med 24, 131-134

Reeves, W C, Hammon, W McD, Doetschman, W H, McClure, H E, and Sather, G, 1955, Studies on mites as vectors of western equine and

Bang, F. B., and Gey, G O, 1952, Comparative susceptibility of cultured cell strains to the virus of eastern equine encephalomyelitis, Bull Johns Hopkins Hosp *91*, 427-461

Barnett, H. C, 1956, The transmission of western equine encephalomyelitis virus by the mosquito *Culex tarsalis* Coq, Am J Trop Med *5*, 86-98

Beaudette, F R, Holden, P, Black, J J, Bivens, J A, Hudson, C B, and Tudor, D C, 1955, Equine encephalomyelitis in pheasants in 1952-1953 Proc 58th Ann Met U S Livestock San Assn, Omaha, 309-321

Buescher, 1955, *Personal communication.*

Casals, J, 1957, The arthropod-borne group of animal viruses, Tr New York Acad Sc (ser *2*), *19*, 219-235

Casals, J, and Theiler, M, 1956, Unpublished work

Casals, J, and Whitman, L, 1957, Mayaro virus a new human disease agent I Relationship to other arbor viruses Am J Trop Med *6*, 1004-1011

Causey, O R, and Maroja, O, 1957, Mayaro virus a new human disease agent III Investigation of an epidemic of acute febrile illness on the River Guama in Para, Brazil, and isolation of Mayaro virus as causative agent Am J Trop Med *6*, 1017-1023

Causey, O R, and Theiler, M, 1958, Virus antibody survey on sera of residents of the Amazon Valley in Brazil, Am J Trop Med *7*, 36-41

Chamberlain, R W, Kissling, R E, Stamm, D D, Nelson, D B, and Sikes, R K, 1956, Venezuelan equine encephalomyelitis in wild birds, Am J Hyg *63*, 261-273

Chamberlain, R W, Rubin, H, Kissling, R E, and Eidson, M E, 1951, Recovery of virus of eastern equine encephalomyelitis from a mosquito, *Culiseta melanura*, Proc Soc Exper Biol & Med *77*, 396-397

Chamberlain, R W, and Sikes, R K, 1955, Laboratory investigations on the role of bird mites in the transmission of eastern and western equine encephalomyelitis, Am J Trop Med *4*, 106-118

Chamberlain, R W, Sikes, R K, and Kissling, R E, 1954, Use of chicks in eastern and western equine encephalitis studies, J Immunol *73*, 106-114

Chamberlain, R W, Sikes, R K, Nelson, D B, and Sudia, W D, 1954, Studies on the North American arthropod-borne encephalitides VI Quantitative determinations of virus-vector relationships Am J Hyg *60*, 278-285

Clarke, D H, and Theiler, M, 1955, The hemagglutinins of Semliki Forest and Bunyamwera viruses, their demonstration and use, J Immunol *75*, 470-474

de Boer, C J, Cadilek, A E, and Walters, S R, 1955, The use of hyperimmune antiserum concentrates in experimental western equine encephalomyelitis, J Immunol *75*, 308-314

Downs, W G, Anderson, C R, and Theiler, M, 1956, Neutralizing antibodies against certain viruses in the sera of residents of Trinidad, B W I Am J Trop Med *5*, 626-641

Dulbecco, R, 1952, Production of plaques in monolayer tissue cultures by single particles of an animal virus, Proc Nat Acad Sc *38*, 747-752

———, 1955, Interaction of viruses and animal cells, a study of facts and interpretations, Physiol Rev. *35*, 301-335

Dulbecco, R, Vogt, M, and Strickland, A G R, 1956, A study of the basic aspects of neutralization of two animal viruses, western equine encephalitis and poliomyelitis virus, Virology *2*, 162-205

Eklund, C M, 1946, Human encephalitis of the western equine type in Minnesota in 1941 clinical and epidemiological study of serologically positive cases, Am J Hyg *43*, 171-193

———, 1954, Mosquito-transmitted encephalitis viruses, a review of their insect hosts and the mechanisms for survival and dispersion, Exper Parasitol *3*, 285-305

Eklund, C M, Bell, J. F, and Brennan, J B, 1951, Antibody survey following an outbreak of human and equine disease in the Dominican Republic caused by the eastern strain of equine encephalomyelitis virus, Am J Trop Med *31*, 312-328

Fastier, L B, 1952, Toxic manifestations in rabbits and mice associated with the virus of western equine encephalomyelitis, J Immunol *68*, 531-541

———, 1954, Infectivity titrations of viruses of western equine encephalomyelitis and Newcastle disease by tissue culture methods, J Immunol *72*, 341-347

Ferguson, F F, 1954, Biological factors in the transmission of American arthropod-borne virus encephalitides, a summary Public Health Monograph No 23, Washington, D C, Government Printing Office, 37 pp

Fothergill, L D, 1939, Human encephalitis caused by virus of equine encephalomyelitis, *in* Third International Congress for Microbiology Report of Proceedings Edited by M Henry Dawson, New York, The Congress, pp 301-302

Frothingham, T E, 1955, Tissue culture applied to the study of Sindbis virus, Am J Trop Med *4*, 863-871

Gajdusek, D C, Anslow, R O, Hubbell, E J, and Yager, R H, 1954, Tissue culture studies of Venezuelan equine encephalomyelitis virus; propagation in human uterine tissue, J Immunol *72*, 224-228

Ginder, D R, and Friedewald, W F, 1951, Effect of Semliki Forest virus on rabbit fibroma, Proc Soc Exp Biol & Med *77*, 272-276

Hammon, W McD, 1948, The arthropod-borne virus encephalitides, Am J Trop Med *28*, 515-525

Hammon, W McD, and Reeves, W C, 1943, Laboratory transmission of western equine encephalomyelitis virus by mosquitoes of the genera *Culex* and *Culiseta*, J Exper Med *88*, 425-434

———, 1946, Western equine encephalomyelitis virus in the blood of experimentally inoculated chickens, J Exper Med *83*, 163-173

———, 1947, Interepidemic studies on arthropod-borne virus encephalitides and poliomyelitis in Kern County, California, and the Yakima Valley, Washington, 1944 Am J Hyg *46*, 326-335

Hammon, W McD, Reeves, W C, and Sather, G,

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14

Arthropod-Borne Group B Virus Infections of Man

The group classification of the arbor viruses is based on antigenic relationship (cf Chap 12), the members of Group B are listed in Table 12 The viruses are subgrouped, in so far as present knowledge permits, according to the closeness of the antigenic relationship existing between the members, although it should be kept in mind that cross-reactions of varying degrees can be demonstrated between all members of the group.

This chapter deals with all of the above viruses except dengue and yellow fever, which are the subjects of Chapters 15 and 16

On the basis of the rather marked antigenic relationship between the viruses of Group B, it is believed probable that all the members stemmed originally from one parent virus and that the individual members represent mutants with somewhat different antigenic and pathogenic properties selected by the pressures of the various host environments to which they have been exposed in the course of their evolution There are many examples, from the laboratory, which illustrate the possibility of selection, by environmental manipulation, of strains of these viruses with modified pathogenic properties The best-known examples are perhaps the adaptation of pantropic yellow fever virus to the brain of the mouse with concomitant loss in viscerotropism for man and the even more successful production, in tissue culture, of the 17D vaccine strain of yellow fever which is almost totally devoid of all pathogenic potentiality for man

TABLE 12 SUBGROUPS GROUP B, ARBOR VIRUSES

Dengue 1 Dengue 2	St Louis Japanese B Murray Valley West Nile Ilheus	Russian Tick-Borne Complex Russian Spring Summer Louping-Ill Central European Tick-Borne Biundulant Meningoencephalitis (Diphasic Milk Fever) Omsk Hemorrhagic Fever Kyasanur Forest Disease	Yellow Fever Uganda S Zika (possibly)

The following members of Group B are not considered at present as members of any particular subgroup Ntaya, Spondweni, Wesselsbron, bat salivary gland virus

St Louis encephalitis viruses in California, Am J Trop Med *4*, 90-105

Reeves, W C, Hammon, W McD, Lazarus, A S, Brookman, B, McClure, H E, and Doetschman, W H, 1952, The changing picture of encephalitis in the Yakima Valley, Washington J Infect. Dis *90*, 291-301

Reeves, W C, Herold, R C, Rosen, L, Brookman, B, and Hammon, W McD, 1954, Studies on avian malaria in vectors and hosts of encephalitis in Kern County, *California II Infection in mosquito vectors* Am J Trop Med *3*, 696-708.

Reeves, W C, Sturgeon, J M, French, E M, and Brookman, B, 1954, Transovarian transmission of *neutralizing substances* to western equine and St Louis encephalitis viruses by avian hosts, J Infect Dis *95*, 168-178

Robinson, M C, 1955, An epidemic of virus disease *in Southern province*, Tanganyika territory in *1952-53* I Clinical features Tr Roy. Soc Trop Med. & Hyg *49*, 28-32.

Ross, R W, *1956*, The Newala epidemic. III The virus, isolation, pathogenic properties and relationship to the epidemic J. Hyg *54*, 177-191

Sabin, A B, 1951, Quantitative studies on prophylactive effectiveness of western equine encephalitis antiserum in mice, *Proc Soc Exper Biol & Med 78*, 655-658

Sanmartin-Barberi, C, 1956, Personal communication

Sanmartin-Barberi, C, Groot, H, and Osborno-Mesa, E, 1954, Human epidemic in Colombia caused by the Venezuelan equine encephalomyelitis virus, Am J Trop Med *3*, 283-293

Schaeffer, M, and Arnold, E H, 1954, Studies on the North American arthropod-borne encephalitides I Introduction Contributions of newer field-laboratory approaches Am J Hyg. *60, 231-236*

Schaeffer, M, Kissling, R E, Chamberlain, R W, *and Vanella, J M, 1954, Studies on the North American* arthropod-borne encephalitides IV Antibody in human beings to the North American arthropod-borne encephalitides Am J Hyg *60, 266-268*

Schlesinger, R W, Morgan, I H, and Olitsky, P K, 1942, Significance of neutralizing antibody in experimental equine encephalomyelitis, J A M A. *119, 618-620*

Shah, K. V, Johnson, H N, Rao, T R, Rajagopalan, P. K, and Lamba, B S, 1957, Isolation of five strains of Sindbis virus in India In preparation

Sharp, D G, 1953, Purification and properties of animal viruses *in* Advances in Virus Research, vol 1, pp 277-313, New York, Acad Press.

Shope, R E, 1953, Antiviral substance from *Penicillium Funiculosum;* effect of helenine upon infection in mice with Semliki Forest virus, J Exper Med *97, 627-638*

Smithburn, K C, 1954, Neutralizing antibodies against arthropod-borne viruses in the sera of long-time residents of Malaya and Borneo, Am J. Hyg *59, 157-163*

———, *1957, Personal communication*

Smithburn, K. C, Kerr, J A, and Gatne, P. B, 1954, Neutralizing antibodies against certain viruses in the sera of residents of India, J. Immunol *72, 248-257*

Smithburn, K C, Mahaffy, A F, and Haddow, A J, 1944, Semliki Forest virus, J Immunol *49*, 159-173

Spence, L P, and Thomas, L, *1958*, Application of hemagglutination and complement-fixation techniques to the identification and serological classification of arthropod-borne viruses' Studies on Chikungunya and Makonde viruses In preparation

Stead, F M, and Peters, R F, 1953, The 1952 outbreak of encephalitis in California Vector control aspects California Med *79*, 91-93

Sulkin, S E, Wisserman, C L, Jr, Izumi, E M, and Zarafonetis, C, *1955, Attempts at experimental* transmission of western equine encephalomyelitis *by means of the chicken mite, Am J* Trop Med *4*, 119-135

Sutton, L S, and Brook, C C, *1954, Venezuelan* equine encephalomyelitis due to vaccination, J A M A *155*, 1473-1476

Syverton, J T, and Scherer, W F, 1953, Applica*tions of strains of mammalian* cells to the study of animal viruses Cold Spring Harbor Symposia on Quantitative Biology *18, 285-289*

Taylor, R M, Hurlbut, H S., Work, T H, Kingston, *J R*, and Frothingham, *T E*, 1955, Sindbis virus a newly recognized arthropod-transmitted virus, Am J Trop Med *4, 844-862*

Trager, W, 1938, Multiplication of the virus of *equine encephalomyelitis in surviving mosquito* tissues Am J Trop Med *18, 387-393*

Victor, J, Smith, D G, and Pollack, A B, 1956, The comparative pathology of Venezuelan encephalomyelitis, J Infect Dis *98*, 55-66

Weinbren, *M P., Kokernot, R H, and Smithburn,* K C, 1956, Strains of Sindbis-like virus isolated from culicine mosquitoes in the Union of South Africa South African M. J *30*, 631-636

Whitman, L, 1956, Personal communication

circumstance of an outbreak with multiple cases, diagnosis of the nature of the epidemic may be obtained by virus isolation from a few cases or by uncomplicated serologic response in a sufficient number to permit presumptive diagnoses of those having the more complex serologic responses It must be borne in mind that new members of the arbor family continue to be isolated and that the presence of Group B antibodies may be attributable to a new agent

In Chapter 12, the epidemiologic implications of the immunologic relationships between the members of Group B are discussed It is believed possible that the presence of one or more members of the group in a given geographic area may, through the production of cross-immunity, act to modify the disease picture caused by or limit the spread of other related viruses It is of interest that members of the closely related subcomplex of St Louis, Japanese B, Murray Valley, West Nile usually seem to occur in a given geographic region in the absence of other members of the complex, St Louis is found in the western hemisphere, Japanese B in the Far East, Murray Valley in Australia and New Guinea, and West Nile in Africa and the Middle East India provides an exception to this rule in that both West Nile and Japanese B have been proved to coexist in one localized area As one application of knowledge of the group relationships, advantage is being taken of the broad spectrum of neutralizing antibodies produced by exposure to more than one member of Group B in an attempt to develop more effective vaccines For references to experimental work bearing on these points, the reader is referred to Chapter 12

ST LOUIS ENCEPHALITIS

(Synonyms American encephalitis used by Japanese and Europeans, SLE as abbreviation)

Introduction

Infection with the virus of St. Louis encephalitis is widespread in central and western United States and produces a varied symptomatology prevailing in the summer and early fall. The disease is similar in many respects to that caused by western equine encephalitis virus and Japanese B encephalitis virus.

History

In the summer of 1932, an epidemic of encephalitis, which was first regarded as von Economo's disease, occurred in Paris, Ill During the following summer, a similar epidemic occurred in and around St Louis and Kansas City, Mo , 1,130 cases were reported in St. Louis County. The disease was then believed to be a nosologic entity different from von Economo's encephalitis. Muckenfuss et al. recovered the causal virus in 1933 by inoculation of infected human brain tissue into monkeys, and in the same year, Webster and Fite obtained similar results by using albino mice as experimental hosts The disease recurred in St Louis County in 1937, in epidemic form involving several hundred persons and since that time has been recognized over a wide area of the United States In some years, only small outbreaks or sporadic cases have occurred, but the disease has been a recurrent problem in certain of the far western states, the last sharp outbreak in California was in 1954 with 99 proved cases (Longshore, 1955). Also, in 1954, one of the largest recorded epidemics of the disease occurred in the lower Rio Grande valley of Texas. There were 373 reported cases, but it is estimated that the actual total was over 1,000 (Chin et al , 1957) Other fairly extensive recent outbreaks have occurred in the lower Ohio River valley in 1955, and in west Texas, in the Grand Junction area of Colorado and in Louisville, Ky , in 1956

Clinical Picture

The description is taken from reports of recent outbreaks of the disease as it occurs today (Lennette and Longshore, 1951; Kokernot et al , 1953; Kunin and Chin, 1957; Ranzenhofer et al , 1957). The clinical description of the early epidemics can be found in previous editions of this volume.

Most cases have a relatively benign course consisting of a brief febrile illness with severe headache, the illness lasting for a few days and being followed by complete and uneventful recovery. In a small percentage of patients, a more progressive illness occurs The onset is characteristically abrupt, with high fever, very severe generalized or localized

The viruses of Group B are capable of causing a variety of disease pictures However, as discussed in Chapter 12, there are certain similarities which are common to all Man is usually an incidental or even tangential (dead-end) host, and the most common result of his infection is the production of a clinically silent or inapparent infection detectable only by the subsequent development of specific antibodies. Certain of the viruses produce their clinical manifestations as a part of a generalized systemic infection Examples are dengue and West Nile fever with their characteristic clinical picture of fever, malaise, rash and lymphadenopathy Other members of the group, in addition to causing inapparent or mild systemic infections, apparently have acquired certain tissue tropisms with the result that, upon occasion, they may involve and damage a specific tissue or organ such as the liver or the brain Yellow fever, St Louis encephalitis and Japanese B encephalitis serve as examples of this type

From the foregoing, it can be realized that the outcome of infection with one of these agents, apart from often being symptom-free, is unpredictable in terms of the severity of the clinical result Even more striking is the fact that the same virus can cause markedly diverse clinical pictures For example, a neuroadapted strain of yellow fever employed as a vaccine has been found to cause a significant incidence of encephalitis rather than liver damage in human recipients (MacNamara, 1953a). West Nile virus, which is usually associated with a denguelike disease picture, produced several cases of encephalitis when given in large doses to humans with advanced malignant neoplasia (Southam and Moore, 1954a) The most diverse array of disease entities has been found to be caused by various strains of the Russian tick-borne encephalitis complex. Although antigenic variation exists between the members of this complex, some investigators feel that all should be considered as strains of one viral entity. Yet, the clinical pictures resulting from infection with one of these strains may be, a fairly mild biphasic febrile reaction with or without meningeal irritation; a severe encephalitis resulting in death or permanent disability; an acute hemorrhagic picture of greater or lesser severity but with no suggestion of CNS involvement (see below).

It should be apparent from the foregoing that the clinical diagnosis of infection with one of the arbor B viruses may be a difficult, if not impossible, task since different viruses may cause the same clinical picture and the same virus may cause different clinical pictures. This, then, becomes a problem which can be resolved only with the aid of the diagnostic laboratory, and even there the problem is not always easy

As a general rule, the most rapid and definitive method of diagnosis is by virus isolation However, this is often impossible or impractical. Therefore, most diagnoses must rest upon serologic evidence. The details of the technics used for serologic diagnosis are given in Chapter 10, and a discussion of the general applications and results is presented in Chapter 12 In the latter chapter, it is stressed that the diagnosis of a Group B infection is frequently complicated by the degree of immunologic cross-reactivity which exists between members of the group The ease and the accuracy with which a serologic diagnosis can be made depends upon the geographic area in which the infection is contracted In the United States, for example, St Louis virus is the only member of the group now known to

(NT) can be used In areas where more than one member of the group infect man, the problem may be more difficult Here, the HI test may give a relatively specific answer or it may reveal only that a Group B infection has occurred, resulting in broadly cross-reactive HI antibodies Depending on the viruses involved, either the CF or the quantitative intracerebral NT may be the diagnostic procedure of choice In regions of high Group B endemicity, where individuals may have had experience with other members of the group prior to the present infection, diagnosis may become quite complex due to the broad serologic response engendered by infection with two related Group B agents If the case is an isolated one, in the absence of virus isolation, diagnosis may prove to be extremely difficult or even impossible. In the more usual

up to 10^{-5} intranasally. Suckling mice are much more susceptible to peripheral inoculation of the virus than are adults This is the basis for the most sensitive type of neutralization test first devised by Olitsky and Harford, 1938, and subsequently applied to St Louis and other viruses by Lennette and Koprowski (1944).

Rats (7 to 8 days old) and wild mice (gray house mice) develop a lethal encephalitis following intracerebral inoculation of the virus. Hamsters, horses and rhesus monkeys are susceptible to intracerebral inoculation with some but not with all strains The rhesus monkey responds by developing fever and signs of CNS involvement but usually recovers and is an unsatisfactory animal for serial propagation of the virus. Cebus monkeys are insusceptible, as are adult rats, sheep, kittens and ferrets

Various animals such as guinea pigs and rabbits develop inapparent infections after inoculation by any route as do monkeys, horses and adult mice after subcutaneous injection with small doses of virus Use is made of this type of reaction in the production of specific immune serum Chickens, ducks and several species of wild birds have been shown to respond to peripheral inoculation of small doses of virus by the development of asymptomatic infections characterized by some degree of viremia The fact that animals in apparent health can harbor virus in the blood which, in turn, may infect insect vectors is considered of prime epidemiologic importance (Hammon et al, 1951a, Chamberlain et al, 1957)

The virus multiplies in the chick embryo after infection by the yolk-sac or the chorioallantoic routes The most commonly used procedure is that of inoculating the chorioallantoic membrane of a 10- to 12-day-old chick embryo. After incubation at 37° C for 3 to 4 days or longer, the egg is opened The membrane is edematous and opaque and exhibits a diffuse proliferation of the ectodermal layers and focal necrosis The virus does not produce definite, discrete pocks or localized lesions but is recoverable from the membrane, the brain and the various viscera and, by the intracerebral mouse test, usually has a titer of 10^{-2} to 10^{-3} As a rule, chick embryos are killed by the virus without specific pathologic changes

A number of investigators have shown that the virus can be propagated in suspended cell cultures of chick or mouse embryo tissue, although cytopathic changes are not observed The virus can multiply in, and in some cases adversely affect, certain types of neoplastic cells. This has been shown both in vivo (Sharpless et al, 1950, Koprowski and Norton, 1950, Koprowska and Koprowski, 1953) and in vitro (Pollard and Bussell, 1952; Scherer and Syverton, 1954). The results of Pollard and Bussell indicate the types of cells which might be useful in tissue culture studies with this virus

Etiology

The basis for placing the virus in Group B of the arbor viruses is an immunologic one as discussed in Chapter 12 It also forms part of a subgroup, the members of which are listed in the introduction to this chapter The diameter of the virus as determined by gradocol membrane filtration is 20 to 30 mμ It passes readily through Berkefeld V and N and Seitz filters. It deteriorates on standing at room temperature, especially at high dilution. This effect can be retarded by the use of a protein diluent such as 10 per cent normal serum or 0.75 per cent buffered bovine plasma albumin It is best preserved by being kept frozen at about −70° C or in buffered 50 per cent glycerol at 4° C or in the lyophilized state In preparing the virus for lyophilization or storage as a frozen suspension, it is best to use undiluted or 50 per cent heated normal serum or a serum fraction such as bovine plasma albumin The virus is susceptible to the action of desoxycholate (cf. Chap 12) and to ethyl ether (Sulkin and Zarafonetis, 1947) which serve to distinguish it from certain other, nonarbor, viruses It is inactivated by 0 1 per cent formalin in from 12 to 18 hours at room temperature (7 to 9 days at 5° C) but not by 1 per cent phenol within at least 25 days Filtered virus is inactivated when held at 56° C for 30 minutes The infective principle is more stable on the alkaline than the acid side of neutrality

Chanock and Sabin (1953) first demonstrated a viral hemagglutinin, most active for erythrocytes of the 1-day-old chick, in infected mouse-brain suspensions The hemagglutinating principle is especially sensitive to pH change but it quite stable at about pH 9

(occipital) headache, malaise, stiff neck and chilly sensations or chills. Nausea and vomiting not infrequently occur In young children, restlessness and irritability are prominent, while in adults, drowsiness, lethargy and some degree of disorientation are common. Convulsions are more common in young children, but they can occur in seriously ill older patients In general, the occurrence of convulsions is a poor prognostic sign, indicating pronounced neurologic damage and greater probability of sequelae Other commonly observed signs and symptoms are tremors, speech difficulties, muscle pains, muscle weaknesses, photophobia and visual disturbances Meningeal irritation is frequently found, as evidenced by nuchal rigidity and positive Kernig and Brudzinski signs In severe cases, stupor, coma or delirium may supervene and may persist for some time One striking observation has been the dramatic suddenness with which remissions can occur leading to complete recovery of patients critically ill at first observation In such cases, recovery is complete within a relatively short time except for generalized weakness and fatigue, which may serve to prolong convalescence. Fever usually lasts from 3 to 10 days, declining gradually In the seriously ill older patient, the illness may be more prolonged with a low-grade fever persisting for 2 to 3 weeks, while in younger patients the febrile phase reaches a higher peak but has a shorter course Although convalescence may be prolonged and marked by generalized weakness, tremors and dulling of intellectual functions, the prognosis for complete recovery is generally good Permanent sequelae, which are uncommon, consist of personality changes, mental deterioration, muscle weakness or frank paralysis. Parkinsonism has not been seen

The white blood count may show either leukopenia or mild leukocytosis, the latter being more common The spinal fluid is under slight pressure and is clear with a pleocytosis of 50 to 250 cells, which are predominantly polymorphonuclear in the early stages and mononuclear later. In young children, the cell count is usually higher and may exceed 500 There is no correlation between clinical severity and height of cell count. Spinal fluid protein is slightly increased, and sugar is normal.

Pathologic Picture

Gross examination of the brain and the cord reveals edema, vascular congestion and small hemorrhages. Microscopic examination shows a cellular infiltration, chiefly of lymphocytes, and engorgement of blood vessels in the meningeal layers. In other tissues of the CNS, there are evidences of acute inflammation, small hemorrhages, focal glial proliferation, and diffuse infiltration of lymphocytes, polymorphonuclear leukocytes, and plasma cells; lymphocytes predominate. The outstanding lesions are the degeneration and necrosis of neurons associated with neuronophagia, exceptionally, small sterile abscesses and areas of necrosis of the gray and white matter occur. Perivascular edema with loculation of the adjoining parenchyma may occur. Perivascular demyelination is not seen

According to Wolf (1950), the relative rarity of permanent neurologic sequelae can be accounted for by the fact that focal necrosis of the tissue is seen far less commonly in St Louis encephalitis than in eastern and western equine and Japanese B encephalitides. However, in areas where western equine and St Louis infections coexist, it has been found impossible to distinguish, in the individual case, between the lesions caused by the two (Finley and Hollister, 1951)

Experimental Infection; Host Range

The experimental animal of choice is the laboratory white mouse It is with this species that Webster in 1937 developed a strain particularly susceptible to St Louis, louping-ill and Russian tick-borne viruses. The strain was designated albino Swiss-W mouse by Casals and Schneider, 1943 Mice can be infected regularly by the intracerebral route, which is the method of choice, although they can also be infected by the intranasal route and occasionally by feeding them virus suspensions, Harford and Bronfenbrenner, 1942 After intracerebral or intranasal infection, an incubation period of from 3 to 4 days is followed by ataxia, ruffled fur, convulsions and paralysis. Prostration and death occur from 1 to 5 days later, and the pathologic picture resembles that seen in the human disease Mice develop the experimental disease when dilutions up to 10^{-7} or 10^{-8} of mouse-adapted virus are given intracerebrally or

nosis of the infection in the United States has not been thought to be complicated by the possibility of infection with related viruses giving serologic cross-reactivity However, it must be kept in mind that an individual may have resided in parts of the world where other Group B virus infections occur. In addition, the recent report of the isolation in Texas of a virus serologically related to St Louis from the salivary glands of insectivorous bats (Burns et al, 1957) raises the question of the possibility of human infections with this agent The evidence available concerning the agent is presented elsewhere in this chapter. Where the possibility of infection, past or present, with a related virus cannot be excluded, diagnosis may be more difficult (cf *Introduction to this chapter and Chap 12*). In such cases, several related viruses or antigens and more than one type of test should be used

Treatment

There is no specific treatment. Symptomatic treatment is indicated, although the severe headache is not relieved by analgesic or even narcotic drugs Good nursing care and general supportive treatment are extremely important for the severely ill patient

Epidemiology

As has been stated earlier, this virus most commonly produces a clinically inapparent infection in man This has been recognized since Wooley and Armstrong (1934) demonstrated NT antibody in 36 per cent of normal individuals following the 1933 epidemic in St Louis. Since that time, a number of studies have shown inapparent infection rates ranging from 10 to 70 per cent of individuals in an endemic or epidemic area (Muckenfuss et al, 1938, Reeves et al, 1952, Ranzenhofer et al, 1957, Chin et al, 1957)

The seasonal incidence of the disease is definite, most cases occur in the late summer and early fall The disease occurs over a wide area of the United States from Kentucky to the Pacific coast in the form of sporadic cases, small outbreaks or rather extensive epidemics It is variable in its appearance from year to year in any one area, but studies of recent epidemics have always revealed the presence of conditions especially or unusually favorable to mosquito breeding (Stead and Peters, 1953, Chin et al, 1957; Ranzenhofer et al, 1957). Distribution of the infection tends to be rural or suburban rather than urban

A recent survey of residents of Trinidad, B.W I., for the presence of NT antibody against various arbor viruses demonstrated its presence against St Louis virus in 4.3 per cent (Downs et al, 1956b). This suggestive evidence for the presence of a heretofore unrecognized virus in that area has been amply confirmed by the isolation of the virus both from mosquitoes (Anderson et al, 1957) and from a nestling wild bird (Downs et al, 1957)

The age distribution of clinical cases has varied in different epidemics In the 1933 epidemic and in the recent ones in Texas and Kentucky, the highest incidence was in persons over 45 years. Longshore et al (1956) state that in California, St Louis virus infection shows no apparent age selection except that it is rare in infants under one year There seems to be no consistent difference related to sex The mortality rate is difficult to assess, since many mild cases escape diagnosis but the case fatality rate appears to have been much lower in recent outbreaks than the 20 per cent figure given for the original epidemic in St Louis (Chin et al., 1957).

The chief vector of the disease in the Far West appears almost certainly to be *Culex tarsalis* (Hammon et al, 1951a; Stead and Peters, 1953) However, the vectors incriminated in the recent Texas and Kentucky outbreaks were *Culex quinquefasciatus* and *Culex pipiens*, respectively (Chin et al., 1957; Beadle et al, 1957, Ranzenhofer et al, 1957) In both cases, the mosquito incriminated was that of the prevalent species, and virus was isolated from naturally infected mosquitoes in pools In the United States, the virus has been isolated from 2 additional species of naturally infected mosquitoes, *Culex stigmatosoma* and *Aëdes dorsalis*, besides the 3 already mentioned (Reeves, 1953). The 3 species from which the virus was isolated in Trinidad were *Culex coronator*, *Culex caudelli* and *Psorophora ferox* (Anderson et al., 1957) In the laboratory, transmission of the virus to animals has been effected by 12 species of mosquitoes from 3 genera: *Culex, Aëdes* and *Culiseta* (Hammon and Reeves, 1943). It seems apparent that the chief mosquito vector varies in different localities, al-

where it maintains its titer on dilution in the absence of added protein (Clarke and Casals, 1955). Inhibitors of the hemagglutinin, which are probably lipid or lipoprotein in nature, occur in serum and in tissue homogenates. Unlike the infective principle, the hemagglutinin is not inactivated by actone or ether, which forms the basis for the preparation of an excellent antigen (Casals and Brown, 1954) Neither the infective nor the hemagglutinating activities are precipitated by protamine sulfate, and a considerable degree of preliminary purification can be achieved by the treatment of virus suspensions with this substance A good hemagglutinating antigen results from such treatment of alkaline suspensions of suckling mouse brain (Warren et al, 1949, Clarke, unpublished results). The relationship of the hemagglutinin to the infective particle is not clear at present

Hemagglutination-inhibiting, neutralizing and complement-fixing antibodies appear in the blood of humans and animals infected with the virus or in animals following vaccination with inactivated virus In general, NT and HI antibodies appear earlier and last longer than do CF antibodies. This difference is important in demonstrating a diagnostic rise in specific antibodies and also in indicating whether detectable antibodies are due to a recent infection or one acquired in the past NT antibody persists for many years if not for life The duration of HI antibody has not yet been determined accurately

The transplacental transmission of NT antibody has been demonstrated in mammals and the transovarian in birds (Smith, 1943, Reeves et al., 1954) The latter observation is believed to be of particular importance due to the significance of birds in the epidemiology of the disease.

Diagnosis

The clinical pictures seen in infections with St. Louis virus are not sufficiently characteristic to permit unassisted diagnosis The mild infections resemble many febrile diseases, and those infections involving the CNS are similar to or identical with CNS infections by a variety of agents. The seasonal incidence is useful in suggesting the possibility of St. Louis infection but is of no value in excluding the two infections most commonly confused with St Louis encephalitis, namely, western equine encephalitis and nonparalytic poliomyelitis A specific diagnosis can be made only by virus isolation or serologic tests

Virus isolation is of little practical value for human diagnosis except in certain fatal cases. The virus has never been recovered from spinal fluid of humans and only very rarely from blood From patients dying early in the disease, it is possible to isolate the virus from CNS tissue. This is best done by inoculation of tissue suspensions intracerebrally into adult or, preferably, suckling mice The brains of animals which sicken or die can then be used for virus identification This is accomplished by use of the same types of procedures as are employed for serologic diagnosis, except that in this case the virus or the antigen constitutes the unknown and is tested against various standard immune sera The details of the procedures used are given in Chapter 10, and a general discussion of their applications to arbor virus infections and Group B virus infections appears in Chapter 12 and in the introduction to this chapter

Accurate diagnosis usually rests on the demonstration of a rise in specific antibodies. For this purpose, two blood specimens are required, the first taken as early as possible in the acute phase, and the second during convalescence, usually about 3 weeks after onset The demonstration of specific antibodies

acquired as the result of the illness rather than an earlier infection Although any of the 3 types of tests (HI, CF or NT) can be used, the CF test has had the most extensive use and is probably the procedure of choice in the United States It is simpler, quicker and less expensive than the NT, and since CF antibody develops more slowly, the acute phase specimen is more likely to be negative or at most to show a low titer of antibody, permitting the demonstration of a clearcut rise in the second, later specimen Finally, since CF antibody does not persist for as long as NT and HI antibodies, results are less likely to be complicated as a consequence of past infection The HI test offers many of the advantages of the CF test except that the antibody develops more rapidly and is more persistent. As has been stated in the introduction, diag-

spread occurrence of the infection throughout the Far East has been recognized only rather recently, mainly by the demonstration of a significant incidence of antibodies in the human and animal population and occasionally by the occurrence of clinical cases

Hayashi, 1934, transmitted the disease to monkeys by the intracerebral inoculation of brain tissue from fatal cases In 1935, a number of other Japanese investigators, including Kasahara, Kawamura and Taniguchi and their associates, isolated the virus from fatal cases and demonstrated neutralizing antibody in recovered cases by using mice as experimental animals

Clinical Picture

The description is based on recent reports of a large number of confirmed cases seen both in the local inhabitants and in American military personnel in epidemic areas (Lewis et al, 1947, Sabin, 1947, Hullinghorst et al, 1951, Dickerson et al, 1952, Lincoln and Sivertson, 1952, Shapoval et al, 1954) Mild or abortive cases have been recognized in which the chief manifestations consist of headache, low-grade fever and malaise of a few days' duration, their incidence is impossible to assess Frank encephalitic manifestations may develop gradually following an initial picture similar to that of the abortive case, or the onset may be abrupt with severe headache and fever rapidly developing into a disabling illness Lincoln and Sivertson (1952) consider nuchal rigidity, headache, fever and sensorial change to be a helpful diagnostic quadriad, others stress the lethargic affect, masklike facies and slow, thick speech, or other speech disturbances Chills and high fever are common in adults, but, although fever is high, chills are said to be uncommon in children Convulsions are common in children but rare in adults Marked flushing of the face, generalized aches and severe anorexia are characteristically seen, and other gastrointestinal disturbances are not uncommon Sensorial change varies from confusion to delirium or coma in severe cases Tremors, including ocular and extraocular motor disturbances, frequently occur Facial paralysis or paralysis of one or more extremity is more common in children, whereas adults most often develop a relatively symmetrical paresis without paralysis or corresponding sensory changes Carpopedal spasm, muscular rigidity or generalized spasticity are found, especially in the more seriously ill patient Abnormal deep and superficial reflexes are frequently observed but are quite variable The fever is sustained, with a relative bradycardia, and reaches its peak in from 2 to 4 days, after which it subsides gradually It is unaffected by antipyretics

The return to an afebrile condition is often followed by dramatic improvement, although the duration of the illness is extremely variable Convalescence is frequently rather prolonged, with persistent generalized weakness, lethargy, in-co-ordination, tremors and nervousness Weight loss may be marked, even in those receiving good hospital care Serious sequelae occur in a significant proportion of cases, but even in such patients the prognosis should be guarded, since slow improvement is possible over a prolonged period The most commonly seen sequelae are mental impairment and personality change Second to these is motor impairment, either of the upper or the lower motorneuron type, resulting in paralyses of greater or lesser extent Less commonly observed are aphasia, cerebellar syndromes, organic psychoses and decerebrate rigidity Japanese B encephalitis is, in general, a more serious disease than St Louis encephalitis with a more protracted course, slower convalescence, a higher incidence of [illegible]
32,000, although the average is around 14,000 There is a moderate neutrophilia and shift to the left The count falls rapidly and returns to normal in the first or second week. The cerebrospinal fluid is usually clear, with normal or slightly elevated pressure and a pleocytosis of 10 to 400 cells, mainly mononuclear Occasionally over 1,000 cells are found, but the degree of pleocytosis is unrelated to the severity of the disease The protein is moderately elevated and remains so for some time as does the pleocytosis, the amount of sugar is normal

Pathologic Picture

Macroscopically, the brain and coverings show edema and congestion, especially in the

though *Culex tarsalis* is particularly efficient owing to the low level of viremia necessary for infection and the fact that it frequently feeds on both avian species and human beings (Reeves, 1953) The role of mites as vectors is at present problematical. The situation as discussed by Reeves and his associates (1955) is as follows Smith et al, 1944, reported the isolation of St Louis virus from chicken mites in nature and, subsequently, the experimental transmission and transovarial passage of the virus by the same mite, other investigators succeeded in isolating the virus from mites parasitic on domestic and wild birds, however, recent extensive field and laboratory studies have failed to furnish convincing evidence for the importance of mites in the epidemiology With the American dog tick, *Dermacentor variabilis*, transovarial passage of the virus through two complete generations has been demonstrated (Blattner and Heys, 1944).

The long-term reservoir of the virus remains unknown, but the role of domestic fowl and wild birds as important sources of vector infection and spread of the virus has been amply indicated by the work of Hammon and Reeves and their associates (Hammon et al., 1951a, Reeves, 1951) who believe that mammals (including man) are not important reservoirs or sources of infection The virus has been isolated from naturally infected wild birds in the United States on 3 occasions and on 1 occasion each in Haiti and Trinidad (Ranzenhofer et al, 1957, Chamberlain et al., 1957, Downs et al, 1957) Studies on the experimental infection of domestic fowl and wild birds have been mentioned previously.

Control

Immunization by means of a formalin-inactivated vaccine was shown to be capable of inducing a high degree of immunity to challenge in mice and to produce detectable neu-
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able and would be impractical for general use because of the cost, the temporary nature of the immunity and the time factor in the face of an epidemic (Reeves, 1951) Since control of the avian sources of vector infection is impossible, control of the vector becomes the only logical approach. As pointed out by Reeves (1951), vector control is practical only if it is economically feasible and is most effective if a thorough knowledge of the principal vector or vectors is available

JAPANESE B ENCEPHALITIS

(Synonyms: Russian autumnal encephalitis, Japanese encephalitis or summer encephalitis; JB, JBE or JE)

Introduction

Japanese B virus infection occurs in a broad band involving all of eastern Asia as far north as the maritime district of Siberia and south well into the East Indies. The band includes the islands off the coast of Asia—Japan, Okinawa, Formosa, Guam, the Philippine Islands and Borneo—and extends westward at least as far as southeastern India (Pond and Smadel, 1954) The disease shows a markedly warm weather seasonal incidence in temperate climates, as in Japan, China and Okinawa, but is not seasonal in warmer regions, such as Malaya It has many features in common with St. Louis and western equine virus infections, including the production of a high proportion of clinically inapparent infections and of a variable clinical picture although, when fully manifest, the CNS involvement in Japanese B encephalitis tends to be the most severe At one time Russian autumnal encephalitis was regarded as a distinct nosologic entity, but later it was shown to be Japanese B encephalitis, Casals, 1944

History

The history of a disease in Japan resembling Japanese B encephalitis goes back at least to 1871, and it has been recognized there as a distinct clinical entity since 1924 when an epidemic occurred involving more than 6,000 cases with an extremely high case fatality rate Since that time epidemics have recurred annually in Japan, although the incidence has varied widely from a small number to over 8,000 cases per year. Epidemics have been recognized in recent years in China, Okinawa and Korea Similar recurrent outbreaks of a seasonal encephalitis have been reported from Formosa, Manchuria and the maritime district of Siberia, but the wide-

mately the same degree of precision Most embryo deaths occur between 48 and 96 hours after inoculation, the virus is found throughout the egg but chiefly in the embryo

The virus can multiply in and in some cases adversely affect certain types of tumor cells in vivo (Sharpless et al, 1950, Koprowski and Norton, 1950, Koprowska and Koprowski, 1953) Haagen and Crodel, 1938, and Kawakita, 1939, showed that the virus could be propagated successfully in Maitland-type tissue culture of chick-embryo cells, and it has been reported to multiply but to produce no consistent cytopathic effect in cultures of chick-embryo fibroblasts, HeLa cells and monkey-kidney epithelium (Scherer and Syverton, 1954, McCollum and Foley, 1957). However, Bhatt and Work (1957) found the virus to produce consistent cytopathic changes after adaptation to both chick-embryo and monkey-kidney epithelial cells McCollum and Foley (1957) report consistent cytopathogenicity for the Detroit-6 cell line in tissue culture without adaptation

Etiology

The immunologic basis for placing the virus in Group B of the arbor viruses is discussed in Chapter 12, the introduction to this chapter lists the other members of the subgroup closely related to Japanese B virus The diameter of the virus has been reported to be 15 to 30 mμ as determined by ultrafiltration through gradocol membranes (Yaoi et al, 1939) The virus passes through Seitz and Berkefeld V and N filters The virus is best preserved by storage frozen at about −70° C, or in buffered 50 per cent glycerol at 4° C, or by lyophilization Satisfactory diluents for preparing the virus for lyophilization or storage as a frozen suspension are undiluted or 50 per cent normal serum, a buffered solution of bovine plasma albumin, or undiluted skim milk adjusted to pH 8 4 Virus dilutions for experimental procedures are best made in 10 per cent normal serum, 0 75 per cent buffered bovine albumin, or 10 per cent skim milk in saline solution The virus is susceptible to the action of desoxycholate (cf Chap 12), which serves to distinguish it from certain other, nonarbor, viruses It is inactivated by 0 2 per cent formalin in the cold without destruction of its antigenicity (Sabin et al, 1943). Filtered virus is inactivated at 56° C. within 30 minutes The infective principle is stable at a somewhat alkaline pH

Sabin and Buescher (1950) reported the occurrence of a hemagglutinin in infected mouse brain suspensions which was demonstrated most satisfactorily with the red blood cells of the 1-day-old chick. The hemagglutinin has the same general properties as does the St Louis viral hemagglutinin (see above) including stability at pH 9 0, resistance to acetone and ether, and the fact that neither the hemagglutinating nor infective activities are precipitated by protamine sulfate

Neutralizing (NT), hemagglutination-inhibiting (HI) and complement-fixing (CF) antibodies develop after infection It has been the experience of American investigators that NT and HI antibodies develop rather rapidly and are usually demonstrable within the first week, while in most cases the CF antibody rises more slowly (Sabin, 1947, Tigertt and Hammon, 1950, Southam, 1956) Soviet investigators, in contrast, report that with their technics CF antibody is detectable within the first week, while NT antibody is not found until the second or third week (Drobyshevskaya, 1954a) Neutralizing antibody endures at significant or high levels for at least 5 years (Buescher et al, 1958) if not for life CF antibody usually reaches a maximum by the third week and declines after 2 months to reach insignificant levels by 5 months (Southam, 1956). In some individuals, however, CF antibody may persist at relatively low titer for at least 5 years (Buescher et al, 1958) HI antibody reaches a maximum by 2 weeks, drops sharply after 5 or 6 months but is still detectable for several years (Southam, 1956).

Transplacental and transovarial transfer of maternal antibody has been demonstrated in mammals and birds In man, such passively acquired antibody disappears by the end of the sixth month(Hale and Lee, 1954) The effect of such antibody in nature is shown by the immunity of newborn horses in epizootics and in the prevention of the viremia in nestling birds, which is a potential source of vector infection

cortical gray matter, basal nuclei, pons and medulla Microscopically are seen perivascular cuffing and meningeal infiltration, chiefly with lymphocytes and some polymorphonuclear leukocytes Neuronal degeneration and necrosis with associated neuronophagia are noted, especially in the substantia nigra, red nuclei, basal ganglia, cerebral cortex, cerebellar cortex and horns of the spinal cord, along with diffuse or focal infiltration of various parts of brain and cord with mononuclear and polymorphonuclear cells The most striking pathologic change is the destruction of the Purkinje cells in the cerebellum, a lesion also observed in the experimental disease and in Russian tick-borne encephalitis In addition, there are patches of encephalomalacia, acellular plaques of spongy appearance in which medullary fibers, dendrites and axons are destroyed, and focal microglial proliferation In chronic cases, focal and diffuse deposition of calcium salts leading to a foreign body response may occur (Zimmerman, 1946) The topography of the lesions in all regions of the cortical gray matter and in the molecular and Purkinje cell layers of the cerebellum is different from that observed in poliomyelitis (Haymaker and Sabin, 1947) More detailed descriptions are given by Zimmerman (1946), Wolf (1950), Tigertt and Hammon (1950) and Lincoln and Sivertson (1952)

St. Louis virus Peripheral inoculation produces an inapparent, immunizing infection

Hamsters develop a fatal infection after intracerebral inoculation but usually show only viremia when infected by extraneural routes. Guinea pigs and rabbits exhibit a silent infection after inoculation by any route, according to recent experience, although earlier results of Japanese investigators indicated that rabbits succumbed to intracerebral inoculation Peripheral inoculation of monkeys, hamsters, guinea pigs and rabbits is a useful procedure for the production of immune serum

A wide variety of domestic animals, domestic fowl and wild birds develop inapparent infections frequently associated with significant viremia This is of epidemiologic significance as providing potential sources of virus for vector infection Horses infected in nature may develop encephalitis, and epizootics among them occur, although the majority have inapparent infections Sows, when experimentally infected early in pregnancy, exhibit a viremia and may produce stillborn or abnormal young, although the dams remain well (Shimizu et al, 1954) In Japan, a high incidence of stillbirths in swine has been associated with outbreaks of Japanese B encephalitis in human beings and horses, and

EXPERIMENTAL INFECTION, HOST RANGE

Japanese B closely resembles the St Louis virus in its effect upon the albino Swiss mouse The intracerebral is the route of choice for inoculation of mice of any age, and mice succumb to the disease when inoculated with dilutions of mouse-adapted virus up to 10^{-8} or occasionally higher Suckling mice are more susceptible to infection by extraneural routes than are older mice, which forms the basis for a highly sensitive type of neutralization test (see above, St. Louis virus) The disease picture in mice is one of a rapidly developing, lethal encephalomyelitis with paralyses, tremors and convulsions beginning in 3 to 8 days after inoculation

Rhesus and cynomolgus monkeys can develop a fatal encephalitis following intracerebral inoculation. The virus can be propagated indefinitely by monkey-to-monkey passage, in which respect this agent differs from

mental infection, a viremia of 2 to 5 days' duration consistently follows the infection of a variety of recently hatched wild and domestic birds. The infection is otherwise silent (Hammon et al, 1951b, Buescher, 1956) At least 2 species of bats, following subcutaneous inoculation, show a nonlethal infection characterized in some instances by very prolonged viremia (Corristan et al, 1956).

Various investigators, including Taniguchi et al., 1936, and Warren and Hough, and Koprowski and Cox, both 1946, showed that the virus can be cultivated in and is pathogenic for embryonated hens' eggs. Smith and Herring (1956) reported that the yolk-sac route of inoculation of 10-day eggs is a more rapid and sensitive method of titration than is the usual procedure of intracerebral inoculation in mice, and that it offers approxi-

shown by serum surveys in Korea and Okinawa (Deuel et al, 1950, Tigertt and Hammon, 1950) and in Malaya and Borneo (Pond et al., 1954, Hale et al, 1956, Federation of Malaya, 1957). Significant or high rates of immunity have also been found in various other parts of continental far-east Asia and on the Pacific islands within the broad endemic band described earlier The recognition of the probable presence of the virus in southern India by antibody survey (Smithburn et al, 1954a) has been confirmed by the diagnosis of a small outbreak of encephalitis in Madras State (Work and Shah, 1956) and the isolation of the virus from mosquitoes in the same area (Work, personal communication)

The occurrence of the disease is confined to the warmer months in temperate regions, and in certain of these areas severe epidemics recur at irregular intervals In warmer climates, cases may occur sporadically and unrelated to season The recent outbreak in India appears to have followed a period of unusually heavy rainfall, resulting in conditions especially favorable for mosquito breeding (Work and Shah 1956)

In some of the Japanese epidemics, a wide age distribution of cases has been seen with more than half of the patients over 50 years of age In other epidemics in Japan, and in Okinawa and Korea, the majority of cases occurred in children (Tigertt and Hamman, 1950, Hullinghorst et al, 1951) Only children were found to have the clinical disease in India (Work and Shah, 1956), and sporadic cases in other areas have been either in native children or in individuals recently entering the endemic region The obvious explanation of these findings lies in the high rates of immunity disclosed by antibody surveys in endemic zones so that only children, or adults recently entering the areas, comprise the population at significant risk There appears to be no relationship between sex and the case distribution The mortality rate is impossible to assess due to the number of inapparent or mild infections which escape diagnosis The case-fatality rate has been reported to be as high as 80 per cent in older age groups, and even in recent outbreaks mainly involving younger patients, the rate has been 35 to 44 per cent (Tigertt and Hammon, 1950, Hullinghorst et al, 1951) Tigertt and Hammon (1950) estimate 28 per cent as the case-fatality rate in United States armed forces personnel in Okinawa, 1945-1949, while in a larger outbreak involving American troops in Korea in 1950, a fatal outcome occurred in 10 per cent of the diagnosed cases (Dickerson et al, 1952) Age and the quality of nursing care are believed to be significant factors in influencing the outcome.

The close association of the epidemic disease with the seasonal incidence of culicine mosquitoes suggested to Japanese epidemiologists in the early 1930's that the infection was mosquito-borne, and in subsequent years, Mitamura and his associates recovered the virus from several *culicine* species, including *Culex tritaeniorhynchus;* and Japanese, Russian and American workers reported the experimental infection of many species of mosquitoes in several genera (reviewed by Hammon, Rees et al, 1949, and Day and Bennetts, 1954) Hammon, Rees et al (1949) confirmed earlier Japanese findings of the experimental transmission of the virus by *C tritaeniorhynchus* and *C pipiens* and reported additional isolations of the virus from naturally infected *C tritaeniorhynchus* (Hammon, Tigertt et al, 1949) Since that time, extensive studies by the scientific staff of the 406 Medical General Laboratory, maintained by the United States armed forces in Tokyo, have indicated that *C tritaeniorhynchus* is probably the sole vector of the human disease in the Tokyo area and perhaps in all Japan as well (Buescher, 1956) Investigation of the Malaysian form of *C tritaeniorhynchus* has shown it capable of experimental transmission of the virus (Hale et al., 1957), but of 10 recent isolations of virus from mosquitoes by the U S Army Medical Research Unit in Malaya only 3 were from this mosquito, while 7 were from *C gelidus* (Federation of Malaya, 1957) The mosquito species found naturally infected in the area of the recent outbreak in India was *C vishnui* (Work, personal communication) It seems probable that the chief vector, or vectors, vary in different areas, and such variations may be of major importance in understanding the different epidemiologic pictures The demonstration by Reeves and Hammon (1946) that the virus can be experi-

Diagnosis

A specific diagnosis can be made only by virus isolation or serologic tests, since the clinical picture, although suggestive in an epidemic, shares many features in common with other febrile diseases and infections of the CNS Virus isolation is of value for human diagnosis only in persons dying early in the disease It is frequently possible to isolate and characterize the virus from CNS tissue in the same manner as for St Louis virus Although Japanese and Soviet investigators have reported virus isolations from the blood of patients, this appears to be a most uncommon finding.

Diagnosis more commonly rests on the demonstration of a rise in specific antibodies as for St Louis virus infection In most of the endemic areas Japanese B is the only Group B virus now known to cause encephalitis in man. However, there is some overlap in the distribution of Japanese B and Russian tick-borne encephalitis in eastern Siberia, and the question of the possible occurrence of both Japanese B and Murray Valley viruses in India has not yet been resolved. Therefore, the problem of the diagnosis of infections with obvious CNS manifestations is, in most areas, somewhat analogous to that of St Louis virus in North America However, outbreaks of dengue have been recognized to occur in many of the same areas as do infections with Japanese B virus, including Japan and Malaya. The possible complicating effect of the co-existence of two related viruses on serologic diagnosis must be kept in mind (see above) In an outbreak of dengue in Malaya, Smith (1957) found that the serologic response as measured by both the CF and HI tests was similar with dengue and Japanese B antigens and considered the NT test the only reliable serologic procedure On the other hand, studies of confirmed cases of West Nile fever in Israel by Goldblum et al. (1957) showed almost equally high titer NT antibodies for West Nile and Japanese B viruses, although there is no reason to believe that the latter infection occurs in that area These investigators found the CF test to be much more specific than the NT test. In an area in India from which both West Nile and Japanese B viruses have been isolated, the CF test proved to be sufficiently reliable to establish the diagnosis of a small outbreak of Japanese B encephalitis (Work and Shah, 1956). In Japan, Korea and Okinawa, the CF test has been found more satisfactory than the NT for the diagnosis of clinical infections Not only is the CF the more practical test, but the slower rise in CF antibody increases the probability that the initial specimen will be negative or show a significantly lower titer than subsequent ones. In some cases, several weeks may be required for the development of CF antibody, and occasionally it may fail to develop at all (Sabin, 1947; Dickerson et al, 1952, Southam, 1956a) Southam (1956a) states that the HI test is uniquely valuable in Japan, since it is diagnostically positive at an earlier time and is as simple as and more reliable than the CF test. Experience with the HI test is limited outside of Japan, but it tends, in general, to be the least specific of the 3 procedures for Group B infections. Therefore, it would appear essential to employ more than one type of test and several related viruses or antigens in establishing a diagnosis whenever past or present infection with a related virus is a reasonable possibility. This is particularly true for the diagnosis of mild infections or for serum survey for the incidence of past infections As discussed earlier and in Chapter 12, the serologic response is extremely complex when an infection is superimposed on past experience with a related virus; a specific diagnosis may then be impossible

Treatment

No specific treatment is available Comprehensive supportive and nursing care are extremely important for all seriously ill patients, and fairly prolonged hospitalization may be necessary during the slow convalescence.

Epidemiology

As is true with St. Louis virus, Japanese B virus produces a high incidence of inapparent infections in man. This has been demonstrated in surveys in Japan by Bawell et al (1950) and by Southam (1956b) From studies on Japanese children, the latter investigator estimates that about 10 per cent become infected annually and that 500 to 1,000 inapparent infections develop for each case of clinically apparent disease Similar high inapparent-infection rates have been

shown by serum surveys in Korea and Okinawa (Deuel et al, 1950, Tigertt and Hammon, 1950) and in Malaya and Borneo (Pond et al., 1954; Hale et al, 1956; Federation of Malaya, 1957). Significant or high rates of immunity have also been found in various other parts of continental far-east Asia and on the Pacific islands within the broad endemic band described earlier The recognition of the probable presence of the virus in southern India by antibody survey (Smithburn et al, 1954a) has been confirmed by the diagnosis of a small outbreak of encephalitis in Madras State (Work and Shah, 1956) and the isolation of the virus from mosquitoes in the same area (Work, personal communication)

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mentally transmitted by 7 species, representing 3 genera, of mosquitoes indigenous to North America indicates the theoretical possibility that the virus could be disseminated in the Western Hemisphere. As to the possible role of mites as vectors, Reeves et al. (1955) failed to obtain experimental evidence for the potential importance of the chicken mite, and they quote the unpublished studies of Hammon and Tigertt in 1953 in endemic areas of Japan in which no virus was isolated from many thousands of mites collected in wild birds' nests

The reservoirs of the virus remain unknown, although the observation of Hurlbut (1950) that experimentally infected mosquitoes can transmit the virus after hibernation at low temperatures for at least 82 days suggests one possible mechanism for survival of the virus during the winter in temperate zone environments. A number of surveys in various endemic areas have shown a high incidence of antibody in the blood of such domestic animals as horses, pigs, cattle, goats, sheep and dogs (Bawell et al, 1950, Tigertt and Hammon, 1950, Hullinghorst et al, 1951, Pond et al, 1954, Drobyshevskaya and Sokolov, 1954) Drobyshevskaya (1954b) reported the isolation of Japanese B virus from the blood of 3 of 280 human beings and 2 of 83 horses in an endemic area during the epidemic season, while a somewhat larger series in the pre-epidemic period was entirely negative. It is of interest that the human donors were and remained well. Despite these observations, it is unlikely that man and domestic animals play an important role in virus dissemination because the high immunity rates and relatively slow population turnovers result in the presence of relatively few individuals capable of developing viremia for vector infection

The probable important epidemiologic role of nestling wild birds, especially of the heron family, in the rapid seeding of the vector population in the Tokyo area of Japan has been well demonstrated (Buescher, 1956). The studies showed a relationship between the instance of viremia in heron nestlings, the frequency of mosquito infection and the occurrence of encephalitis in human beings and animals Serum surveys have shown a high or significant incidence of antibody in the blood of a variety of species of wild birds (Hammon et al, 1951b; Buescher, 1956; Smorodintsev et al., 1954b). Since the wild-bird population turnover is rapid, there is available annually a large number of new susceptible nestlings, and these birds consistently develop a viremia of several days' duration following infection. The wild bird appears to be of importance as a disseminator of both St. Louis and Japanese B viruses, and this also appears to be true of the Murray Valley virus Of probable significance is the fact that quite unlike the situation with both St. Louis and Murray Valley viruses, the domestic chicken appears to escape infection with Japanese B virus completely, although it is readily infected experimentally.

One peculiar epidemiologic observation is that extensive dissemination of the virus can occur among domestic animals in endemic regions of Japan in the absence of human epidemics and, conversely, epidemics have occurred without associated epizootics (Sabin et al, 1947, Burns et al., 1949, Buescher, 1956) This suggests the presence of at least two different epidemiologic cycles which can operate quite independently.

CONTROL

The main attempts to control the disease have been by means of formalin-inactivated vaccines Vaccination has been practiced by the Russians since 1941, using a mouse-brain preparation In the United States, a formalinized mouse-brain vaccine was developed by Sabin et al, 1943, and Warren and Hough, and Koprowski and Cox, both 1946, reported on the preparation of an inactivated vaccine from infected chick embryos Smadel et al (1947) described the chick embryo vaccine subsequently used in the Far East Both types of vaccine were used extensively from 1945 to 1951 for immunization of U S armed forces personnel and for field trials involving many thousands of Japanese school children The results of the field trials are reviewed briefly in a recent article by Tigertt and Berge (1957) in which references are given to more detailed reports on evaluation of the vaccine. The study was handicapped because no large outbreaks of the disease occurred during the period of investigation, but the final cautious estimate was that the results indicated some degree of protection This was

based both on serologic studies and on a lower incidence of the disease in the vaccinated as compared with the control series for the entire study period Unmodified cases did occur among vaccinated children Evaluation of the results of vaccination of U S personnel was difficult to assess, but the effectiveness of the vaccines was considered sufficiently dubious that routine vaccination was discontinued in 1951. So far, efforts directed toward the development of a more potent and stable vaccine have met with no success (Pond and Smadel, 1954)

Vector control offers a logical approach when it is feasible Much more needs to be learned of the epidemiology of the disease in each endemic area before practical programs of control can be achieved

MURRAY VALLEY ENCEPHALITIS

(SYNONYM. Australian X Disease)

INTRODUCTION

Australian investigators appear to agree generally that Murray Valley encephalitis and Australian X disease are one and the same, and they will be so considered here. Although the opinion has been expressed that Murray Valley virus could be a variant strain of Japanese B, sufficient evidence is available to justify its designation as a distinct entity within the subgroup described earlier in the chapter, even though a close immunologic relationship exists between the Murray Valley and Japanese B viruses

HISTORY

During the summer of 1917-1918 an epidemic of acute encephalitis broke out in Australia, involving 134 persons of whom half were less than 5 years of age, 70 per cent succumbed to the disease, Cleland and Campbell, 1917 The disease reappeared in 1922, 1925 and 1926 in milder form but not again until 1951 A virus was isolated from the brains of 3 patients by Cleland and Campbell, 1917, 1919 but was lost before a comparative study of its properties could be made These early outbreaks were designated Australian X disease

In 1951 an epidemic of encephalitis again occurred in eastern Australia in the valleys of the Murray and the Darling Rivers in Victoria and New South Wales There were 40 recognized severe cases of which more than half were children, and there were 17 deaths Later studies showed a high rate of mild or inapparent infection. The disease was named Murray Valley encephalitis, and the virus was isolated from the brains of several fatal cases by French (1952) and by Miles et al (1951). The evidence for considering Australian X disease and Murray Valley encephalitis to be identical is discussed by Burnet (1952), and the serologic studies of McLean and Stevenson (1954) lend support to the concept There have been no reported epidemics since 1951

CLINICAL AND PATHOLOGIC PICTURES

Clinically and pathologically, the disease closely resembles that following Japanese B virus infection (Robertson and McLorinan, 1952, Robertson, 1952) Cases vary from a mild illness to severe and fatal encephalitis The disease tends to be more severe in the very young The onset is typically abrupt, with malaise, headache, fever and irritability Lethargy, drowsiness and gastro-intestinal manifestations occur early, as do convulsions in young children In severe cases cerebration and consciousness become progressively impaired, this occurs rapidly in children but more gradually in adults Cervical rigidity is a constant finding Improvement may occur at any stage and lead to rapid and complete recovery if it occurs early, but frequently the disease is progressive Tremors, involuntary movements, pareses, paralyses and respiratory arrhythmia are features of the severe illness The most common residua are impairment of cerebral function, paralyses and personality changes In fatal cases death may occur early or after prolonged illness The cerebrospinal fluid shows a moderate pleocytosis ranging from 20 to 750 cells, averaging around 100 Protein may be slightly elevated

The primary pathologic change is neuronal damage with secondary degeneration of nerve fibers Neurons throughout the entire CNS are involved, destruction of Purkinje cells being especially striking The spinal cord changes closely resemble those of poliomyelitis (Garven and Margolis 1952). The proliferative and infiltrative response resembles that of other arbor virus encephalitides. The

lesions are both diffuse and localized. In chronic stages of the disease areas of total necrosis are seen, and deposition of calcium salts may be found

Experimental Infection; Host Range, Etiology, Diagnosis

Two out of 3 of the initial Murray Valley virus isolations by French (1952) were successful only by chorio-allantoic membrane inoculation of chick embryos, and the presence of virus was recognized only by the occurrence of a few pocks on the membrane. The third isolation was successful, both in chick embryos and by intracerebral inoculation of mice. French suggests that the demonstration of pocks on the chorio-allantoic membrane is the most sensitive indicator of the presence of the virus and can yield positive results when virus cannot be demonstrated by other technics

The virus is highly pathogenic for chick embryos inoculated by the amniotic, the allantoic and the yolk-sac routes, and death occurs in 3 days with characteristic pathologic changes and the occurrence of virus in various tissues and fluids It is also highly pathogenic for adult and suckling mice by the intracerebral route and for suckling mice by extraneural routes The picture in mice is characterized by convulsions, in-co-ordination, tremor and paralysis of the hind limbs. The titer of virus in the brain is high, and it is readily transmitted serially (French, 1952).

The rhesus monkey develops encephalitis and succumbs after intracerebral inoculation but shows no reaction following subcutaneous inoculation. Rabbits and guinea pigs show essentially silent, immunizing infections, except for a febrile response in the latter, after inoculation by any route (French, 1952, Miles, 1952) The virus readily produces a fatal infection in hamsters on extraneural as well as intracerebral inoculation, in which respect it resembles West Nile virus and differs from Japanese B and St. Louis viruses (Hammon and Sather, 1956) Various domestic animals, fowl and wild birds are susceptible to infections with the virus and may succumb following intracerebral inoculation. Most frequently, the response to peripheral inoculation consists of the development of viremia and subsequently of antibodies (French, 1952; Miles, 1952). The young chick is particularly susceptible to infection and regularly develops a viremia following peripheral inoculation; the infection is otherwise silent (McLean, 1953a). It provides a useful tool for entomologic studies and is believed to be of epidemiologic importance as a source for vector infection.

The immunologic basis for placing the virus in Group B is discussed in Chapter 12; the other members of the subgroup closely related to Murray Valley virus are indicated above. Australian investigators recognized a close relationship between Murray Valley and Japanese B viruses but considered them to be distinct The detailed studies of Pond and his associates (1955) confirm the lack of identity between the two and provide evidence that the virus endemic in southeast Asia is Japanese B and not Murray Valley. The virus is 20 to 50 mμ in diameter by gradocol membrane filtration (Miles, 1952) The conditions for preservation of the virus are the same as those described for St. Louis and Japanese B viruses MacDonald (1952) described a hemagglutinin in saline extracts of infected suckling mouse brain which was demonstrable with erythrocytes of the pigeon or the day-old chick. McLean (1956) prepared the hemagglutinin by acetone-ether extraction of suckling mouse brain according to the procedure of Casals and Brown (1954) The infective and hemagglutinating properties can be partially purified by protamine sulfate treatment as described for St Louis and Japanese B viruses

Neutralizing (NT), complement-fixing (CF) and hemagglutination-inhibiting (HI) antibodies develop after infection, although the HI test has not yet been applied to the study of natural infections As is true with other members of the group, the CF antibody is less enduring than the NT and has been used as an indicator of recent infection (Anderson et al, 1952, Miles, 1955; Miles and Dane, 1956)

Specific diagnosis rests upon virus isolation from CNS tissue or upon demonstration of the development of homologous antibodies in association with the disease. The principles are the same as those outlined for St Louis and Japanese B infections Since extensive epidemics of dengue have occurred in the same

general areas as do Murray Valley infections, the problem of serologic diagnosis resembles that of Japanese B (see above) The group relationships make the interpretation of serum surveys for past infection especially difficult, for example, Miles and Dane (1956) recognize the possibility that their surveys in the Northern Territory of Australia may have been complicated by the presence of dengue infection but give reasons for believing that this was not the case

Treatment; Epidemiology, Control

There is no specific treatment.

Murray Valley virus has the arbor virus characteristic of producing a high incidence of inapparent infections in man and infects a high proportion of horses without producing evidence of disease (Anderson et al, 1952; Miles and Howes, 1953). It is estimated that inapparent infections in man are 500 to 1,000 times more common than clinically demonstrable cases (Anderson et al, 1952, Burnet, 1952) This estimate is identical with that given by Southam for Japanese B virus infections in Japan (see above) Although all ages may be attacked, the clinical disease is seen in children with particular frequency A fatal outcome is obviously uncommon in relation to total infection rates, but the diagnosed case fatality rate is high

Outbreaks of the disease have occurred in the Murray-Darling basin in Victoria and New South Wales, but on the basis of serologic surveys, the infection is believed to be widely distributed throughout eastern Australia and endemic in the Northern Territory and Queensland (Burnet, 1952, Miles and Howes, 1953) Studies in both 1952 and 1954 revealed that 80 to 90 per cent of the aborigines in certain areas in the Northern Territory have antibody to the virus, and it is suggested that this virus may be the cause of some of the sporadic cases of severe encephalitis seen there (Miles and Dane, 1956) Earlier serologic studies suggested the presence of the virus in New Guinea, and recently it was isolated from the brain of a fatal case of encephalitis (French et al, 1957) Due to group relationships, it is still not clear whether or not Murray Valley virus infections have occurred in India (Smithburn et al, 1954a) The studies of Pond et al (1955) indicate that the neutralization of Murray Valley virus by human sera from Malaya and Borneo can be attributed to previous infection with Japanese B virus

The virus has not been isolated from naturally infected mosquitoes, but attempts have not yet been made during the peak of an epidemic Experimental infection of and transmission by various mosquito species is discussed by McLean (1953b; 1957), who also presents evidence for the belief that *Culex annulirostris* is the principal vector.

The following concept of the epidemiology of the disease is based on discussions by Anderson (1953), Anderson and Eagle (1953), Miles and Howes (1953), and Miles and Dane (1956) The infection is probably endemic in the Northern Territory, Queensland and perhaps northern New South Wales where the virus is believed to be maintained throughout the year by a wild bird-mosquito cycle When meteorologic conditions are especially favorable, the infection may break out of the endemic areas and be brought into the southeast by a chain of migratory bird-mosquito-bird cycles Once introduced into the southern epidemic region, the domestic fowl probably plays an important role in human disease as a source of vector infection

A striking correlation has been observed between the years of excessive rainfall in the Northern Territory and Queensland and the subsequent appearance of epidemics in the south It is suggested that such meteorologic data should give warning of the imminence of future outbreaks (Miles and Howes, 1953) Control measures might then be instituted, although the lack of a vaccine and inadequate knowledge of the chief vectors make practical control problematical

WEST NILE FEVER

Antibody surveys indicate that West Nile virus infection may be widespread in Africa, including East, West and Central Africa and the plateau region of South Africa It is a highly endemic infection in Egypt and has been discovered to be the cause of recurrent epidemics of a denguelike disease in Israel It occurs in India, as has been demonstrated both by antibody surveys and virus isolation

The virus was first isolated from the blood of a native of Uganda who was suffering from

a mild febrile illness, Smithburn et al, 1940 Later, it was isolated from the blood of 3 Egyptian children by Melnick et al (1951) Subsequently, isolations were made by Taylor et al (1956) from human blood, mosquitoes and birds in Egypt. Bernkopf et al. (1953) first diagnosed the epidemic disease in Israel by virus isolation from a patient.

Clinical Picture

The following description is drawn from reports of recent epidemics in Israel by Bernkopf et al (1953), Goldblum et al. (1954) and Marburg et al (1956) Mild, atypical and abortive cases are known to occur The clinically recognizable infection is a self-limited, nonfatal disease which closely resembles dengue fever The incubation period is 3 to 6 days. The onset is always sudden, and the characteristic findings are fever, severe headache, rash and generalized lymphadenopathy The rash is pinkish, discrete and maculopapular, occurring mainly on the trunk and not followed by desquamation Other common manifestations are malaise, generalized myalgias, ocular pain, gastro-intestinal disturbances, flushed face, injected conjunctivae and reddening and soreness of the throat In some cases, a biphasic temperature curve is seen with a recurrence of symptoms in milder form in association with the secondary temperature rise The blood shows a leukopenia with a relative lymphocytosis and shift to the left In a very small percentage of cases, slight transitory meningeal involvement occurs, and in such cases the cerebrospinal fluid shows a pleocytosis and increase in protein However, West Nile virus is an uncommon cause of aseptic meningitis in Israel The acute phase lasts, as a rule, for 3 to 5 days, with extremes of 1 to 12 days There have been no fatalities and no sequelae Recovery is more rapid in children than in adults, who may show persistent weakness and fatigue for one to several weeks

Southam and Moore (1954a) reported on the use of recently isolated strains of West Nile virus for therapeutic trials in patients with advanced malignant neoplasia Nine of 78 patients had definite or suggestive evidence of diffuse encephalitis attributable to the virus infection. These observations coupled with the occasional occurrence of meningeal involvement in the course of natural infections indicate that the virus is potentially capable of infecting the human CNS

Experimental Infection; Host Range

West Nile virus affects common laboratory animals in a manner similar to the related viruses described above. The albino Swiss mouse is highly susceptible to intracerebral inoculation, and lethal encephalitis ensues after 3 to 8 days The titer of virus in the brain is of the order of 10^{-8}, and the virus is readily maintained by serial passage Suckling mice are much more susceptible to peripheral inoculation than are adults The virus causes encephalitis and death in rhesus monkeys on intracerebral inoculation but only produces fever and immunity following intravenous administration It immunizes but is not pathogenic for rabbits and guinea pigs Hamsters are susceptible to both intracerebral and peripheral inoculation, in which respect the virus resembles Murray Valley and differs from St. Louis and Japanese B viruses (Hammon and Sather, 1956) Various domestic animals experience inapparent immunizing infections with low levels of viremia in some instances (Taylor et al., 1956).

The domestic chicken and pigeon and various wild birds can be infected by peripheral routes and develop viremia of varying degree (Work et al, 1955; Taylor et al, 1956) The infection may or may not be fatal The susceptibility of the chicken is related to age, young chicks readily acquire the infection and circulate the virus in fairly high titer In young chicks of at least one strain, successful infection by any route has led invariably to a fatal outcome (Whitman, personal communication) Older chickens become partially or completely refractory to infection West Nile resembles the related viruses in the importance of avian species in the epidemiology of the infections it induces.

Watson, 1943, Taylor (1952), and Fendrich et al (1957), described cultivation of the virus in chick embryos. Death of infected embryos occurs with such regularity that the virus can be titrated in eggs as well as in mice Taylor found the yolk-sac route of inoculation to be simple and satisfactory, but Fendrich et al considered the intravenous the route of choice. Bernkopf et al. (1953) observed

pocks on the membrane following chorio-allantoic membrane inoculation but did not find this a satisfactory method either for initial virus detection or virus titration

The virus adversely affects or is oncolytic for a variety of tumors, both of man and lower animals, grown in experimental animals (Sharpless et al, 1950, Koprowski and Norton, 1950, Toolan and Moore, 1952; Koprowski, 1956, and Moore, 1957) When administered to human beings with advanced malignant neoplasia, the virus was never curative but produced, in some cases, a transitory inhibitory effect on tumor growth (Southam and Moore, 1952).

The virus can be grown in Maitland type of tissue culture, Lennette and Koprowski, 1946. It is regularly cytopathogenic for HeLa cells, chick embryo fibroblasts and monkey kidney epithelial cells in tissue culture (Scherer and Syverton, 1954, Bhatt and Work, 1957) and grows, with variable cytopathic effects, in a variety of other cell lines (Moore, 1957).

Etiology and Diagnosis

The basis for the classification of the virus in Group B has been discussed in Chapter 12 and in this chapter. The diameter of the virus is 20 to 30 mμ, as determined by gradocol membrane filtration (Smithburn and Bugher, 1953) It has the same properties as do St Louis and Japanese B viruses, and the same conditions apply to its preservation (see above). A hemagglutinin in saline extracts of infected mouse brain was first reported by Sabin, 1951, and subsequently in acetone-ether extracted suckling mouse brain by Casals and Brown (1954) The hemagglutinin also has the same properties as do those of related viruses Neither infective nor hemagglutinating activities are precipitated by protamine sulfate (Warren et al, 1949, Clarke, unpublished results)

Neutralizing (NT), complement-fixing (CF) and hemagglutination-inhibiting (HI) antibodies develop following infection Goldblum et al (1957) in a study of over 300 patients in Israel found that CF antibody was first demonstrable in the second week, reached a maximum at 2 to 3 weeks and began to decline after 7 months to reach very low levels at 2½ years They report that NT antibody was detectable in only half the cases in 2 to 3 weeks, although it was present in high titer in all at 2 to 3 months and persisted at a high level for at least 2½ years These investigators observed serious loss in both CF and NT antibodies on storage of specimens in a commercial deep freeze In a study of induced infections in man, Southam and Moore (1954b) observed no marked differences in the rate of development of CF, NT and HI antibodies, which were all usually detectable 3 to 4 weeks after infection They considered all 3 technics to be equally satisfactory

Accurate diagnosis rests on virus isolation or the demonstration of a rise in specific antibody in association with the infection The clinical features of the disease do not sharply delineate it from other entities, and the disease has been mistaken for dengue Unlike infections with members of the Group previously discussed, virus isolation is frequently a practical diagnostic procedure When repeated blood specimens were obtained during the early phase of the disease, as many as 38 per cent of cases have been so diagnosed, and 77 per cent when a specimen was secured on the first day (Goldblum et al, 1957) The technic for virus isolation and identification is similar to that for St Louis virus (see above) except that the specimen consists of blood serum rather than CNS tissue Virus has not been isolated from spinal fluid, throat swabs or stools following natural infection When virus isolation fails, diagnosis rests on the demonstration of a rise in specific antibodies as described for St Louis infection The problem is complicated by the occurrence of group and subgroup relationships, and the use of more than one type of test and several related viruses or antigens is indicated if past or present infection with a related virus is a reasonable possibility Goldblum et al (1957) found that confirmed cases of West Nile fever develop NT antibodies to approximately equal titer against West Nile and Japanese B viruses, and Taylor et al (1956) reported that similar sera from Egypt neutralized both Japanese B and Ntaya viruses Goldblum found the CF test much more reliable than the NT The HI test is even less specific than the NT, but when performed under controlled conditions with a variety of related antigens,

the homologous titer is found to be, in general, higher than the heterologous A tentative diagnosis can be made from the HI pattern of response, but confirmation is required by other procedures (Casals and Theiler, personal communication)

TREATMENT, EPIDEMIOLOGY, CONTROL

Treatment is entirely symptomatic.

Present knowledge of the epidemiology of infections with West Nile virus comes from two different sources Israel, where the disease is recurrently epidemic, and Egypt, where the infection is highly endemic and the disease goes unrecognized as a clinical entity In Israel, inapparent infection is unrelated to age as determined by serologic studies, and virus has been isolated from the blood of individuals who were at no time ill (Bernkopf et al, 1953; Goldblum et al., 1954; Goldblum et al, 1957)

In both Egypt and Israel, the infection is highly seasonal, and it is estimated that it is the cause of two thirds to three quarters of the "summer fevers" in Israel. There is evidence for the occurrence of occasional infection during the winter in the highly endemic areas of Egypt (Taylor et al, 1956, Goldblum et al, 1957). The virus has been isolated from man in Uganda, Egypt and Israel, and from mosquitoes and birds in Egypt A single isolation from *Culex molestus* mosquitoes is also recorded from Israel (Tahori et al, 1955). In India, its presence was first indicated by a significant incidence of NT antibody (Smithburn et al, 1954a), and later it was isolated from mosquitoes (Work, personal communication). In addition to the above localities, NT antibody has been reported to occur in the Sudan, Kenya, the Belgian Congo, Nigeria and the Union of South Africa (Smithburn and Jacobs, 1942, Smithburn, 1952; Dick, 1953, Kokernot et al, 1956)

The early acquisition of immunity in the endemic areas of Egypt causes West Nile fever to be a disease of early childhood there (Melnick et al, 1951, Taylor et al, 1956). In Israel, the case rate is highest in infants, but all ages are involved. There is no apparent influence of sex on the incidence of infection

Experimental transmission of the virus by mosquitoes was reported by Philip and Smadel, 1943, by Kitaoka, 1950, by Davies and Yoshpe-Purer, 1954, and by Hurlbut (1956) in the course of extensive investigations on West Nile infection in arthropods. Apart from these studies and the clinical and immunologic observations reported from Israel, most of the information regarding the epidemiology of the disease stems from the extensive studies in Egypt by Taylor et al (1956). Similar studies have not yet been reported from Israel, although there is some evidence that *Culex molestus* may be a vector there (Tahori et al., 1955). A concept of the ecology of the virus in Egypt was arrived at by virus isolations from man, birds and mosquitoes, by serologic studies in man, birds and other animals and by experimental infection of birds and other animals and of various arthropod species. The dominant cycle by which the virus is propagated is believed to be bird-mosquito-bird with *Culex univittatus* the principal vector. Some evidence was obtained that the virus may overwinter by means of a slow continuous cycle in which *Culex pipiens* is involved Human infection is widespread in those areas where mosquitoes prevail and occurs seasonally in relation to the presence of virus in mosquitoes Although viremia in man may be sufficient to infect mosquitoes, it seems unlikely, in view of the high immunity rate in the population, that a man-mosquito cycle is of importance. A wide range of other vertebrates is infected by the virus, but their infections are believed to be tangential or "dead-end."

Protection from, or control of, the vectors offers the only present approach to control of the disease

ILHEUS VIRUS INFECTION

Ilheus virus has been found in South and Central America and in Trinidad, B.W.I. It has been isolated from mosquitoes in Brazil, Honduras and Guatemala, and from both mosquitoes and man in Trinidad Serologic surveys indicate it to be a prevalent human infection in the Amazon valley in Brazil and in parts of Trinidad. This is not to imply that the virus does not occur elsewhere, but information is not available from other areas. It seems probable that the virus is limited to the Western Hemisphere.

Laemmert and Hughes (1947) first isolated the virus from forest mosquitoes in Brazil. Anderson et al. (1956) recovered the virus from forest mosquitoes in Trinidad, and further mosquito isolations have been reported from Honduras and Guatemala (de Rodaniche, 1956, de Rodaniche and Galindo, 1957) The virus has been recovered from human blood in Trinidad (Downs et al, 1956a, Anderson, Delpeche and Downs, personal communication).

Information on the clinical picture is meager. With regard to the two natural infections in Trinidad, one was in a patient bled on the second day of illness who subsequently developed an encephalitic reaction and was very ill for 2 weeks The other was a completely asymptomatic infection in a mosquito collector who had been working in the forest Infection of patients with advanced malignant neoplasia was tried by Southam and Moore (1951) for possible therapeutic effect, using mouse-brain adapted virus Of 9 successful infections, 6 were asymptomatic, but in 3, mild encephalitis ensued On this basis, the virus can be considered to have the characteristics of its group in producing both inapparent and clinically demonstrable reactions in man

The virus has the same properties as do the other members of the group Its subgroup relationship is given earlier in this chapter Its diameter by filtration is 18 to 26 mμ (Smithburn and Bugher, 1953) The albino Swiss mouse is the laboratory animal of choice, and the effect of age on susceptibility is the same as for related viruses. Other common laboratory animals, in general, show no visible reaction to but are immunized by the virus The virus multiplies in but does not kill the chick embryo unless massive doses are given, and it multiplies well in Maitland-type tissue culture Marmosets, some marsupials, and rodents develop somewhat sustained viremia after infection, which is of possible significance in dissemination of the virus For more detailed accounts, the reader is referred to reports of Koprowski and Hughes (1946), de Rodaniche (1956) and Taylor (1952) The virus parasitizes and in some cases adversely affects certain animal tumors in vivo (Koprowski and Norton, 1950, Koprowski, 1956) It grows in a variety of cell lines in tissue culture with variable cytopathogenicity (Moore, 1957) A hemagglutinin was described in acetone-ether extracted suckling-mouse brain by Casals and Brown (1954) and is readily demonstrable in alkaline aqueous extracts of such tissue. The latter preparation is improved by treatment with protamine sulfate, which precipitates neither infective nor hemagglutinating activities (Clarke, unpublished results).

The principles for the diagnosis of recent or past infection with the virus are the same as for related viruses and are discussed in Chapter 12 and in this chapter All 3 types of antibody, NT, CF and HI, develop following infection.

Some of the virus isolations from mosquitoes have been from lots containing 2 or more genera However, 3 of the 9 isolations were from lots made up of mosquitoes of the *Psorophora* genus only, in one case, only *Psorophora ferox* and in another mainly this species Laemmert and Hughes (1947) demonstrated experimental transmission of the virus by *P ferox* and by two *Aëdes* species. The probable importance of *Psorophora* species, in particular *P ferox*, as vectors is suggested The recent isolation in Guatemala was from *Sabethes chloropterus*

On the basis of serologic surveys in Trinidad, human infection with the virus appears to be common in lowland forested areas (Downs et al, 1956b) In a similar survey in the Amazon valley of Brazil, a high incidence of human infection was also indicated (Causey and Theiler, 1958). In both surveys, a significantly higher rate was found for males than for females, which is attributed to the more intimate contact of the former with the forest

RUSSIAN TICK-BORNE COMPLEX

Introduction and History

The viruses making up the Russian tick-borne complex will be treated as different strains of one virus and considered together despite the diverse clinical pictures resulting from infection Such a treatment was suggested by Pond et al (1953) as a result of [illegible]

hoped to clarify what is, at present, a confusing and poorly understood subject. Ulti-

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TREATMENT, EPIDEMIOLOGY, CONTROL

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milk from infected goats (Smorodintsev et al., 1954a). The resultant infection has been called biundulant meningo-encephalitis or diphasic milk fever by different workers; the nomenclature is further confused by the use of the former name for the clinically indistinguishable disease resulting directly from tick-bite. The clinical picture in man following infection with any of the above virus strains shows considerable variation from case to case, but, from strain to strain, the major difference seems to lie in the severity and the degree of CNS involvement, the louping-ill strain showing the lowest pathogenicity for man, and the Far Eastern Russian, the highest. An obvious analogy can be found in poliomyelitis with which, in fact, these infections are often confused.

At the present time two additional strains of the complex are known which cause a clinical picture quite at variance with the above in that CNS involvement apparently does not occur, but the systemic phase of the infection may be quite severe and accompanied by hemorrhagic manifestations. The first recognition of this type of disease was in the Omsk Oblast in Siberia in 1944-1946, hence it is known as Omsk hemorrhagic fever, and the causative virus was isolated in 1947 by an expedition of Soviet scientists under Chumakov (cf. Gajdusek, 1953). A somewhat similar disease has just been recognized and the virus isolated in Mysore state, India, by Work et al. (1957). It has been called Kyasanur forest disease and is believed to be of recent origin in the region, at least as the cause of serious or fatal infections in the human and monkey population. The relationship between the Siberian and Indian viruses and diseases has yet to be assessed.

Clinical Pictures

In the following discussion, aspects of the clinical pictures which are common to all strains are considered first. Subsequently, those infections which can lead to CNS involvement are discussed, and finally the hemorrhagic forms are described. The clinical pictures caused by the viruses of the Russian tick-borne complex are characteristically protean in nature, and the complex might be thought of as the prototype of all arbor viruses, since the diverse results of infection embody essentially all of those seen in one or another of the other arbor virus diseases. Infection may be inapparent, so mild as to escape diagnosis without laboratory aid, moderate to severe with or without permanent residua, or fatal. Onset of symptoms is abrupt, and a diphasic disease is probably characteristic, although one or the other phase may be absent or unrecognized. The first phase consists of a generalized systemic infection marked by fever, severe headache, malaise, generalized pains and gastro-intestinal manifestations. It has frequently been described as influenzalike. Signs of meningeal irritation may be observed but at this stage represent meningismus rather than true meningitis. The blood characteristically shows a leukopenia. The end of the first phase is marked by a fall in temperature and subsidence of symptoms. After a variable period, the onset of the second phase is marked by return of fever and with neurotropic strains, the development of signs of CNS involvement. The blood picture changes to show a moderate leukocytosis in infections involving the nervous system. This phase is comparable with clinically recognizable infection in St. Louis, Japanese B and other encephalitides. It must be emphasized that one or the other phase may not be noted.

Louping-ill infections in man may consist only of the first influenzalike phase or may have a diphasic course with a mild or severe meningo-encephalitis in the second phase. The diphasic picture is a feature of the disease in sheep. A favorable outcome without sequelae is to be expected and suggests that this strain has a decreased pathogenicity for man. The above account is based mainly on the reports of Edward (1948) and Lawson et al. (1949).

The tick-borne encephalitis now widely distributed throughout western Russia and Central Europe has been described under a variety of names by Soviet, Czechoslovakian, Yugoslavian and Austrian investigators (Silber and Soloviev, 1946; Smorodintsev et al., 1954a; Davidenkov et al., 1954; Rugevich et al., 1954; Chumakov, 1955; Hloucal and Gallia, 1949; Krejci, 1949; Bedjanič et al., 1955; Grinschgl, 1955). Among others, the names given have been western form of Russian tick-borne or spring-summer encephalitis, biundulant meningo-encephalitis, diphasic milk fever and Central European (or Czechoslo-

TABLE 13 GEOGRAPHIC DISTRIBUTION OF DISEASES CAUSED BY RUSSIAN TICK-BORNE COMPLEX

Area	Diseases
British Isles	Louping-ill, predominantly a disease of sheep
Soviet Union from the Far East to Western Russia in Europe	Russian tick-borne or spring-summer encephalitis Biundulant meningo-encephalitis or diphasic milk fever Omsk hemorrhagic fever
Central Europe, extending from Sweden and Finland in the north into the Balkan Peninsula in the south	Central European tick-borne virus encephalitis (Czechoslovakian, etc , tick-borne encephalitis)
India	Kyasanur forest disease
Malaya	Not known but virus isolated from ticks

mate clarification awaits the acquisition and exchange of more information by interested investigators throughout the world

The members of the complex have the common property of being transmitted by ticks of the family *Ixodidae* Mosquito transmission has never been demonstrated, and the available evidence indicates that they fail to multiply in such hosts However, the decision to consider all of the agents as strains of one virus rests on comparative antigenic analyses of all but one member of the complex. The missing member is the virus of Kyasanur forest disease, an entity isolated too recently to have allowed detailed study Although some antigenic differences are detectable, especially in detailed analysis by cross complement-fixation test, the overwhelming body of evidence indicates that no consistent distinction can be established among the strains by reciprocal neutralization tests and vaccination challenge experiments (Casals and Webster, 1944; Edward, 1950; Pond et al., 1953, Pond and Russ, 1955; Konowalchuk et al., 1957). Extensive comparative studies with convalescent human sera following natural infection have not been reported, although sera obtained from human beings vaccinated with a Russian spring-summer strain gave a similar degree of protection of mice against louping-ill, a Czechoslovakian strain and 4 Russian spring-summer strains (Pond et al, 1953) Since the experimental animal host seems to be unable to distinguish immunologically between the viruses, it seems reasonable to assume that man reacts in the same way, and on this basis the classification rests. It is probable that variations produced by different environmental conditions in nature influence the behavior of the individual strains, including their pathogenicity for man.

The present known distribution of the various diseases is given in Table 13.

In the British Isles, louping-ill has been known for over a century as a disease of sheep capable of producing CNS manifestations, the viral etiology was established in 1930 by Pool et al in Scotland It has never been a serious cause of human disease, most of the reported infections having occurred in laboratory workers, but a few natural infections have been described in persons working in close association with sheep in enzootic areas In the Soviet Union, the disease now known as Russian tick-borne or spring-summer encephalitis was first recognized as a clinical entity in the Far Eastern region by Panov in 1934, and Silber and his associates isolated the causative virus in 1937 However, Soviet scientists trace the disease back at least to 1880, when Kozhevnikov described a peculiar form of epilepsy now believed to be a sequel to tick-borne encephalitis Subsequent to the recognition of the disease in the Far East, it was also found to be present in Siberia and European Russia but in a generally less severe form, which led Soviet investigators to distinguish between the Far Eastern and Western varieties The disease now widespread in Central Europe appears to be indistinguishable from the Western variety. It is impossible to say how long the virus has been present in Central Europe, but recent reports suggest that it is becoming an increasingly important public health problem there Of considerable epidemiologic significance is the recognition by Soviet scientists that infection may be acquired by drinking

infection. Convalescence is prolonged in both, but there are no sequelae Kyasanur forest disease appears to be the more serious infection and more often fatal

Pathologic Picture

Detailed descriptions of the pathologic changes caused by neurotropic strains can be found in the review by Silber and Soloviev (1946) and the recent article by Grinschgl (1955). The chief distinction between the picture produced by the tick-borne complex and those of St Louis and Japanese B encephalitis is that the cervical cord is more often affected in the former In the individual case, poliomyelitis cannot be distinguished pathologically The leptomeninges are swollen, the brain is congested, and petechial hemorrhages are seen, especially in the brain stem, the medulla, and the horns of the spinal cord All stages of neuronal degeneration and necrosis are found, mainly in the gray matter, involving the anterior horns of the spinal cord, the medulla, the pons, the Purkinje cells of the cerebellum, and the cerebral motor cortex Extensive infiltrative and proliferative reactions occur Infiltration and degeneration of peripheral nerves, hyperplasia of the spleen and parenchymatous degeneration of the liver, the kidney and the myocardium have been reported

Detailed reports of pathologic findings in Omsk hemorrhagic fever are not available, but from the account of Gajdusek, gross hemorrhages in the gastro-intestinal tract and fine hemorrhages elsewhere are encountered The basic lesion is believed, by Soviet investigators, to involve the capillaries The pathologic picture in Kyasanur forest disease is based only on descriptions of 2 human cases by Work et al The main changes consisted of focal necrosis and bile stasis in the liver and acute tubular degeneration in the kidney The lungs showed patchy consolidation, and in one of the cases there was extensive oozing of blood into the lung in association with massive gastro-intestinal hemorrhage

Experimental Infection, Host Range

Of the common laboratory animals, the albino Swiss mouse is the most useful All of the strains are pathogenic for this animal by the intracerebral route on primary isolation with the exception of the virus of Omsk hemorrhagic fever. It is not clear whether the use of suckling mice might eliminate the need for adaptation passages with this strain. All of the strains, except for louping-ill, are also highly pathogenic for mice by other routes, although the intracerebral is preferable for most purposes The distinctive property, within Group B, of pathogenicity for adult mice on extraneural inoculation is useful in virus characterization Regardless of the strain used, the picture in the mouse is a fatal encephalomyelitis, the virus is present to high titer (10^{-8} to 10^{-9}) in the brain and can be maintained readily on serial passage

Strains of the complex with neurotropic potentialities for man characteristically produce encephalitis on intracerebral inoculation into rhesus monkeys, although a fatal outcome is not invariable Inoculation by peripheral routes may result in viremia and a silent, immunizing infection in the rhesus monkey but with one Russian strain, the cynomologus monkey was found to be more satisfactory for the production of consistent and sustained viremia It is suggested that this reaction provides a useful method for vaccine assay (Morris et al, 1955) The infection in this animal is otherwise silent, although encephalitis follows initial infection by the intracerebral route The virus of Omsk hemorrhagic fever produces a febrile reaction and immunity in the rhesus monkey following intraperitoneal inoculation, but no information is available as to results by other routes The high degree of pathogenicity of the Kyasanur forest disease virus for 2 species of Indian monkeys is indicated by the occurrence of epizootics among the langur (*Presbytis entellus*) and bonnet (*Macaca radiata*) monkeys in association with the human epidemics (Work et al, 1957)

The guinea pig may show a febrile reaction to various strains, and Soviet investigators report encephalitis in a small percentage of animals receiving neurotropic strains. It is not a satisfactory animal for virus propagation, although it provides a source of immune serum Some neurotropic strains are pathogenic for hamsters Rabbits are either insusceptible to infection with members of the complex or develop a silent infection with viremia of short duration

vakian, etc) tick-borne encephalitis. The incubation period is usually 7 to 10 days. The diphasic aspect of the infection is marked, although an unknown, probably large, number of cases never progress beyond the first nonspecific illness The duration of the first phase is from 5 to 10 days, and the afebrile period is usually 4 to 10 days Intense headache and high fever usher in the second phase, and the most frequent type of CNS involvement is serous meningitis of varying degree of severity Other CNS manifestations consist of spinal, bulbospinal and ascending types of paralyses and of encephalitis. The incidence of these complications varies considerably. The prognosis in those cases showing only meningitis or mild meningo-encephalitis is the most favorable Pareses or paralyses are generally of the flaccid type and may be transient or permanent. Death or permanent sequelae more commonly occur in those developing paralyses, and the gravest outlook is for those with the bulbospinal form Paralysis and atrophy of the muscles of the neck and the shoulders (shoulder-girdle paralysis) are not common Nystagmus, vertigo, somnolence and visual and mental disturbances indicate the development of encephalitis which, in severe cases, may go on to delirium or coma In all types of the disease, even in those without frank meningeal signs, the cerebrospinal fluid shows a moderate pleocytosis and increase in protein The usual duration of the second phase is 8 to 12 days

Convalescence is quite prolonged and marked by headache, debility and weakness Residual paralyses are the most common sequelae, but their incidence is not high According to Soviet sources, 3 to 5 per cent of the cases with paralyses show a progressive chronic course, including Kozhevnikov's epilepsy. The latter is characterized by constant clonic spasms of specific muscle groups which from time to time increase in intensity and develop into an epileptic seizure.

The first descriptions of serious human disease due to the viral complex were of the Russian Far Eastern form of encephalitis. The following is based mainly on the review by Silber and Soloviev (1946) The main differences between the preceding pictures and the Far Eastern variety are in the gravity of the latter disease and the absence of a diphasic course. Abortive and moderately severe cases were seen which showed serous meningitis with or without mild encephalitis, but many severe cases occurred in which the most characteristic finding was encephalitis accompanied by involvement of the cervical cord and bulbar centers. In such patients, the case fatality rate was high, and those surviving usually showed neck and shoulder-girdle paralysis of the flaccid type. Other permanent neurologic or psychotic sequelae without parkinsonism were seen, as were epileptiform seizures of the Kozhevnikov type. A convalescent period of several months was usual even for the uncomplicated case. It is not clear whether this severe form of the disease is still commonly found in parts of the Soviet Union, or whether this now represents the extreme disease picture with any of the neurotropic strains

Details concerning the two hemorrhagic forms of the infection are not yet available The following account is based on the review of Omsk hemorrhagic fever by Gajdusek (1953) and the preliminary report of Kyasanur forest disease by Work et al (1957) The two are considered together, although their relationship is not yet known. The incubation period in Kyasanur forest disease is 5 days or less, and both diseases frequently have a diphasic course, the second phase consisting of a return of fever and recurrence of previous symptoms without CNS involvement Both have a sudden onset with fever, headache and gastro-intestinal manifestations. Vomiting and diarrhea may be marked in the Indian disease Characteristic or even diagnostic of both is a hemorrhagic injection or enanthem of the palate. Generalized lymphadenopathy is common in both, and meningismus not uncommon, but true meningitis is absent Epistaxis, hematemesis and uterine bleeding are common but seldom profuse in Omsk hemorrhagic fever, while in the Kyasanur disease, epistaxis and severe gastro-intestinal hemorrhage occur in some cases. The latter disease may occur as a moderate or severe systemic disease without hemorrhagic manifestations Marked leukopenia and thrombocytopenia are seen in both, but the urine is normal in the Siberian disease and frequently shows albumin, casts and leukocytes during the febrile period of the Indian

the development of hemagglutination-inhibiting antibody following natural infection except for Kyasanur forest disease in which it has been observed to develop more rapidly than does the CF antibody (Work et al, 1957). Neutralizing antibody can be detected earlier than CF by use of the sensitive intraperitoneal technic, and in milder infections, a significant number of patients may fail to develop detectable CF antibody at any time (Smorodintsev et al, 1954a, Drobyshevskaya et al, 1954) Neutralizing antibody has been shown by Soviet investigators to persist for 20 years or more following infection with Russian strains, while CF antibody is of shorter duration

Diagnosis

A clinical diagnosis may be indicated by the following features of the diseases the sharply seasonal aspect in temperate climates, a history of contact with forested areas or of tick-bite, a history of drinking raw milk from goats, a diphasic disease course, the characteristic injection or enanthem on the palate in Omsk hemorrhagic fever and Kyasanur forest disease However, many other infections may be confused with these diseases, in particular, poliomyelitis with the severe CNS infections, other infectious hemorrhagic diseases in Siberia and bacterial enteritis in India Accurate diagnosis must rest on virus isolation or serologic study In infections with neurotropic strains, virus isolations have been made during the acute phase from blood and cerebrospinal fluid and from the brains of early fatal cases Numerous isolations have been made from blood early in the course of Omsk hemorrhagic fever and Kyasanur forest disease The technics have been mentioned in the section on St Louis and West Nile viruses Isolations of Omsk hemorrhagic fever virus were made in both guinea pigs and mice (Gajdusek, 1953), and Verlinde et al (1955) used the chick embryo inoculated on the chorio-allantoic membrane for some of the Austrian strain isolations

For serologic diagnosis, paired serum specimens are required as for St Louis virus The neutralization and complement-fixation tests have been used extensively for serologic diagnosis of all of these diseases In many areas where members of the complex occur there is no information to suggest that other members of Group B cause human infections, exceptions are India and the Far Eastern region of the Soviet Union Therefore, either neutralization or CF test usually give reliable results, although it should be kept in mind that Soviet investigators report a number of patients with relatively mild infections who failed to develop CF antibody In the Far Eastern Soviet Union, Japanese B infections also occur, but the serologic relationship between the two viruses is such that differentiation should be clear by one or the other test except in an individual previously infected with one and currently infected with the other, here a complex serologic response would be expected Results from the study of Kyasanur forest disease indicate that the CF test can provide a specific diagnosis but also suggests that other Group B infections occur in the same area leading to the diagnostic difficulties seen with multiple Group B infections (Kulkarni et al, 1958) HI antibody produced by this Indian strain is broadly cross-reactive within the group and is only of tentative diagnostic value The HI test should be of aid in the diagnosis of infections in Central Europe and most of the Soviet Union

Treatment

Treatment of any of the infections is symptomatic Some Soviet investigators appear to be convinced that convalescent human or hyperimmune horse or goat serum can have a beneficial effect if given early in infections with neurotropic strains (Chumakov, 1955) Hospital care is important for seriously ill patients as are measures to combat the effects of blood and fluid loss in the Omsk and Kyasanur forest diseases

Epidemiology

The capacity of strains of the complex to produce a high proportion of inapparent infections in man has been revealed by the demonstration of neutralizing antibody in a large part of the healthy population of endemic areas in the Soviet Union (Silber and Soloviev, 1946, Alekseyev and Gulamova, 1954) The sharply seasonal, spring-summer-autumn, character of outbreaks has been an aspect of all epidemiologic studies, the peak incidence in various areas depending on the seasonal activity of the vector tick species of

Sheep are susceptible to both louping-ill and Russian strains of the virus. Although slight differences in the clinical pictures have been described, both regularly produce a fatal encephalitis after intracerebral injection The louping-ill strain produces a variable picture from inapparent infection to fatal encephalitis following peripheral inoculation, but it is not clear that Russian strains have been shown to cause encephalitis by extraneural routes The preceding is based on reviews by van Rooyen and Rhodes (1948) and by Silber and Soloviev (1946).

Smorodintsev et al. (1954a) demonstrated the presence of virus in the milk of both naturally and experimentally infected goats and designated the relatively milder infections in man seen with this strain as biundulant meningo-encephalitis (diphasic milk fever). Recently, van Tongeren (1955-1956) has shown that the same phenomenon can be produced experimentally in goats with an Austrian strain capable of producing the most severe type of CNS damage in man The goat shows no marked reaction to infection but develops viremia and hemorrhages into the udder as indicated by the appearance of small amounts of blood in the infected milk

Infection of a large variety of other animals has been demonstrated with one or more members of the complex, many such infections are silent, but others lead to severe or fatal illness. In addition to sheep and goats, the results of studies of small mammals, especially forest rodents, and of wild birds are of particular significance Earlier investigations in the Soviet Union are reviewed by Silber and Soloviev (1946), and an important contribution has been made recently by Levkovich et al. (1955) who studied various animals and birds after experimental infection by tick bite or subcutaneous inoculation using a Russian strain They demonstrated sustained viremia in mice, voles, hedgehogs, ground squirrels, chipmunks and certain wild birds as well as in sheep and goats, hence, any or all of these animals are potential sources of vector infection and may act as disseminators of virus An Austrian strain has been shown to produce viremia lasting from 3 to 10 days in chickens, sparrows and rabbits (Pattyn and Wyler, 1955). A local muskrat species and two small field rodents have been found highly susceptible to the virus of Omsk hemorrhagic fever, and various other animals and wild birds develop a silent infection following experimental inoculation or tick bite.

Strains of the complex have been shown to grow in embryonated hens' eggs and to cause death of the embryos Verlinde et al (1955) described characteristic pocks after chorio-allantoic membrane inoculation of an Austrian strain Russian spring-summer and louping-ill strains grow in and destroy a variety of animal tumors in vivo without necessarily causing death of the host (cf Moore, 1954) Both these strains can be cultivated in the Maitland type of tissue culture and both show marked cytopathogenicity for HeLa cells in tissue culture (Oker-Blom, 1956, Moore, 1957) The Russian strain also grows in other cell lines, but complete cytopathogenicity was observed only with HeLa cells and a line of human adult fibroblasts (Moore, 1957). Similar information is lacking for other strains of the complex

Etiology

The immunologic basis for placing this virus complex in Group B has been discussed in Chapter 12, and justification for considering all members of the complex as strains of one virus is given above This position may be strengthened or altered as more information becomes available. The diameter of the Russian spring-summer and louping-ill strains is 15 to 25 mμ by gradocol membrane filtration. All strains have been shown to pass through Berkefeld V and N or Seitz filters Various strains have shown much the same properties as do the members of Group B discussed earlier, and the optimal conditions for preservation and experimental work are similar. Hemagglutinating antigens have been prepared with the Russian spring-summer and Kyasanur strains by acetone-ether extraction of suckling mouse brain or by protamine sulfate treatment of alkaline aqueous extracts of the same tissue (Casals and Brown, 1954; Kulkarni et al, 1958). Such hemagglutinins are most active with the red cells of the 1-day-old chick or the adult goose

Neutralizing and complement-fixing antibodies develop following infection with all strains. No information is available concerning

the development of hemagglutination-inhibiting antibody following natural infection except for Kyasanur forest disease in which it has been observed to develop more rapidly than does the CF antibody (Work et al., 1957). Neutralizing antibody can be detected earlier than CF by use of the sensitive intraperitoneal technic, and in milder infections, a significant number of patients may fail to develop detectable CF antibody at any time (Smorodintsev et al., 1954a, Drobyshevskaya et al., 1954). Neutralizing antibody has been shown by Soviet investigators to persist for 20 years or more following infection with Russian strains, while CF antibody is of shorter duration.

Diagnosis

A clinical diagnosis may be indicated by the following features of the diseases: the sharply seasonal aspect in temperate climates, a history of contact with forested areas or of tick-bite, a history of drinking raw milk from goats, a diphasic disease course, the characteristic injection or enanthem on the palate in Omsk hemorrhagic fever and Kyasanur forest disease. However, many other infections may be confused with these diseases; in particular, poliomyelitis with the severe CNS infections, other infectious hemorrhagic diseases in Siberia and bacterial enteritis in India. Accurate diagnosis must rest on virus isolation or serologic study. In infections with neurotropic strains, virus isolations have been made during the acute phase from blood and cerebrospinal fluid and from the brains of early fatal cases. Numerous isolations have been made from blood early in the course of Omsk hemorrhagic fever and Kyasanur forest disease. The technics have been mentioned in the section on St. Louis and West Nile viruses. Isolations of Omsk hemorrhagic fever virus were made in both guinea pigs and mice (Gajdusek, 1953), and Verlinde et al. (1955) used the chick embryo inoculated on the chorio-allantoic membrane for some of the Austrian strain isolations.

For serologic diagnosis, paired serum specimens are required as for St. Louis virus. The neutralization and complement-fixation tests have been used extensively for serologic diagnosis of all of these diseases. In many areas where members of the complex occur there is no information to suggest that other members of Group B cause human infections; exceptions are India and the Far Eastern region of the Soviet Union. Therefore, either neutralization or CF test usually give reliable results, although it should be kept in mind that Soviet investigators report a number of patients with relatively mild infections who failed to develop CF antibody. In the Far Eastern Soviet Union, Japanese B infections also occur, but the serologic relationship between the two viruses is such that differentiation should be clear by one or the other test except in an individual previously infected with one and currently infected with the other, here a complex serologic response would be expected. Results from the study of Kyasanur forest disease indicate that the CF test can provide a specific diagnosis but also suggests that other Group B infections occur in the same area leading to the diagnostic difficulties seen with multiple Group B infections (Kulkarni et al., 1958). HI antibody produced by this Indian strain is broadly cross-reactive within the group and is only of tentative diagnostic value. The HI test should be of aid in the diagnosis of infections in Central Europe and most of the Soviet Union.

Treatment

Treatment of any of the infections is symptomatic. Some Soviet investigators appear to be convinced that convalescent human or hyperimmune horse or goat serum can have a beneficial effect if given early in infections with neurotropic strains (Chumakov, 1955). Hospital care is important for seriously ill patients, as are measures to combat the effects of blood and fluid loss in the Omsk and Kyasanur forest diseases.

Epidemiology

The capacity of strains of the complex to produce a high proportion of inapparent infections in man has been revealed by the demonstration of neutralizing antibody in a large part of the healthy population of endemic areas in the Soviet Union (Silber and Soloviev, 1946, Alekseyev and Gulamova, 1954). The sharply seasonal, spring-summer-autumn, character of outbreaks has been an aspect of all epidemiologic studies, the peak incidence in various areas depending on the seasonal activity of the vector tick species of

the areas The geographic distribution of the various diseases is given above (Table 13), and it is clear that one or more members of the complex are known to be present over an extremely wide area of Europe and Asia. The first epidemic recognized in central Europe was in Czechoslovakia in 1948 (Hloucal and Gallia, 1949). Subsequently, similar outbreaks were reported from Yugoslavia, Austria and Poland (Kmet et al., 1955; Richling, 1955, Przesmycki et al, 1956). New foci of Omsk hemorrhagic fever were recognized recently, although it is not known as an entity outside of western Siberia (Gagarina and Netskii, 1955) Antibody to the complex was found in a few individuals in India well before the recent outbreaks of Kyasanur forest disease (Smithburn et al, 1954a), and there is suggestive evidence for the occurrence of antibody in man in Malaya where a strain of the complex has been isolated from ticks (Smith, 1956)

Since these diseases are believed to result directly or indirectly from infected tick bites, they are rural rather than urban in distribution, and the case distribution according to age, sex and occupation is determined by the closeness of contact with sources of infection, in particular, with forested areas Thus, a high proportion of infections is usually seen in young adult male agricultural and forest workers, although cases have occurred among urban dwellers of all ages and either sex following visits to rural areas, and milk-borne infections have a familial or group aspect Infection with the louping-ill strain may be acquired as a result of handling tick-infested sheep and has been seen in abattoir workers

Statistics as to case fatality rates are extremely variable. The highest figure, 29 per cent, is given for the early epidemics in the Far Eastern Soviet Union Subsequent outbreaks in Western Russia and Central Europe have been associated with rates from 0 to 14 per cent No fatal human infections with the louping-ill strain have been reported The over-all mortality from Omsk hemorrhagic fever is given as 1 to 2 per cent, while preliminary reports from India suggest that in Kyasanur forest disease, the rate may be as high as 28 per cent The explanation for these differences is unknown, but they suggest a considerable degree of variation in pathogenicity for man from strain to strain.

In the Soviet Union, the two major tick vectors of the neurotropic virus strains are *Ixodes persulcatus* and *I. ricinus* *I. persulcatus* tends to be an eastern species and *I. ricinus* a western, but the distribution of the two overlaps, and where this occurs the seasonal incidence of disease may be prolonged due to differences in seasonal activity of the tick species. Repeated virus isolations have been made from naturally infected ticks of both species (Silber and Soloviev, 1946; Smorodintsev et al., 1954a). *I. ricinus* is the only vector implicated elsewhere in Europe, and virus has been isolated from wild-caught ticks of this species in Czechoslovakia, Hungary, Yugoslavia, Poland and Austria (Rampas and Gallia, 1949; Fornosi and Molnár, 1954, Likar and Kmet, 1956; Przesmycki et al., 1956, Jettmar, 1956). Both *Dermacentor pictus* and *D marginatus* have been found infected with Omsk hemorrhagic fever virus (Gajdusek, 1953, Gagarina and Netskii, 1955) and in India, Kyasanur forest disease virus has been recovered repeatedly from wild-caught *Haemaphysalis* ticks, the species so far identified being *H spinigera* (Work and Trapido, personal communication). The strain of the complex isolated in Malaya was obtained from *Ixodes granulatus*. Transmission of infection to experimental animals has been demonstrated with the louping-ill strain by *I. ricinus*, with Russian spring-summer strains by *I persulcatus* and *I. ricinus* and with Omsk hemorrhagic fever by *D pictus*. Unlike the purely vector role of the mosquito in other virus infections, the tick can be both vector and reservoir, for in studies carried out with Russian strains, it has been found to remain infective for long periods of time and to transmit virus transovarially through successive generations (Silber and Soloviev, 1946). Transovarial transmission has also been demonstrated with Omsk hemorrhagic fever in *D. pictus* (Gajdusek, 1953) The tick acts as the indirect vector in the milk-borne infections recently recognized by Soviet investigators (Smorodintsev et al, 1954a) So far, such infections have been related only to the ingestion of raw milk from goats infested with ticks in pasture It is believed that a similar

epidemiologic picture occurs in Austria (van Tongeren et al., 1955-1956)

Certain small mammals, in particular rodents, and some wild birds have been implicated in the epidemiologic cycle of these viruses by virus isolation, demonstration of antibody or both. In early studies, such vertebrates were considered to be the reservoirs, but more recently they have come to be looked upon rather as virus disseminators which serve to increase the density of tick infection in a given area and spread it into more distant ones. In the Soviet Union, virus or antibody has been found in squirrels, chipmunks, hares, field and forest mice, wild rats, voles, hamsters, hedgehogs, porcupines, moles, grouse and various Passerine birds (Chumakov et al., 1940, Pavlovsky, 1941, Silber and Soloviev, 1946; Warren, 1946). Antibody has been found in wild rodents in Malaya (Smith, 1956) and in India (Work and Trapido, personal communication). Many of the same animals and birds show sustained viremia after experimental infection.

The importance of sheep and goats in certain of these diseases has been discussed. Cattle and horses apparently experience immunizing infections but have not been implicated otherwise.

CONTROL

A formolized mouse brain vaccine has been used in the Soviet Union for some years and has given favorable results in a number of field studies (Warren, 1946. Gajdusek, 1953). A similar vaccine prepared from sheep cord and brain has been successful in protecting sheep from louping-ill infection (van Rooyen and Rhodes, 1948). Soviet workers also recommend immune serum prophylaxis for nonimmune individuals following tick-bite in endemic areas or laboratory exposure. The use of protective clothing and insect repellents and, where feasible, local tick and rodent control measures are advocated. According to Soviet sources, one acaricide aerosol bomb can exterminate most of the ticks in 5 to 7½ acres of forest and inhibit tick repopulation for a period of at least 5 to 7 days (Chumakov, 1955). Control of tick infestation of sheep by dipping can be used in the prevention of louping-ill infections. Goats' milk should be boiled where milk-borne infection is suspected.

UGANDA S VIRUS

Uganda S virus was isolated from *Aëdes* mosquitoes in Uganda by Dick and Haddow (1952). Subsequently, another strain of the virus was isolated by Ross (1956) and characterized as identical with Uganda S by Spence and Thomas (1958). This strain was isolated from mosquitoes in Tanganyika and designated Makonde virus. Uganda S virus has not been associated with overt human disease, although neutralizing antibodies have been demonstrated in the sera of human beings in various areas.

The virus multiplies in *Aëdes aegypti* mosquitoes infected by inoculation (Whitman, personal communication). It is highly pathogenic for the albino Swiss mouse in which it produces a lethal infection by the intracerebral route in adult animals and by both intracerebral and intraperitoneal routes in suckling mice. It is readily maintained by serial passage in mice and attains a brain titer of 10^{-7} to 10^{-8}. It shows little or no pathogenicity for other common laboratory animals including the embryonated egg, although it multiplies in the latter host (Taylor, 1952). Tissue culture studies have shown poor multiplication and cytopathogenicity in various cell lines (Moore, 1957). The virus is of the same size (15 to 22 mμ) as other Group B viruses (Smithburn and Bugher, 1953), and the optimal conditions for storage and experimental work are the same. Studies carried out by Kerr (1952) and by MacNamara (1953b) have shown a close immunologic relationship between Uganda S and yellow fever viruses demonstrable both by complement-fixation and neutralization tests. In the latter, the relationship tends to be more marked when yellow fever immune serum is tested against Uganda S virus. A hemagglutinin was demonstrated in acetone-ether extracted suckling mouse brain by Casals and Brown (1954).

Neutralization of the virus has been observed with human sera obtained in various parts of the world. A significant incidence of neutralizing antibodies has been found in Uganda, Tanganyika, India, Malaya and

must be interpreted with caution, since when comparative studies were done, the same sera were usually shown to neutralize at least one other Group B virus, and MacNamara showed that convalescent sera from proved cases of yellow fever neutralized Uganda S virus It has not been determined unequivocally that this virus can cause human infection, although a closely related but not identical virus has been isolated from the blood of a febrile child in Natal, South Africa (South African Institute for Medical Research, 1956)

ZIKA VIRUS INFECTION

Zika virus was isolated by Dick et al (1952) from the blood of a febrile sentinal monkey in a forest in Uganda and later from wild-caught *Aedes africanus* mosquitoes in the same forest. It was subsequently isolated from the blood of a febrile patient in Nigeria by MacNamara (1954) The virus has been shown to be capable of producing a clinically demonstrable infection in man in 3 cases studied by MacNamara and in the experimentally induced infection reported by Bearcroft (1956). The infection in man is associated with fever, headache and malaise, and possibly jaundice in some, although the last may have arisen from concomittant infection with another agent

The virus is readily maintained by serial passage in the albino Swiss mouse in which it produces a fatal infection and brain titers of around 10^{-7} following intracerebral inoculation. The suckling mouse is highly susceptible to infection by the intraperitoneal route Guinea pigs and rabbits show no visible reaction to infection, although they may be immunized, and a nonfatal infection is likewise produced in the chick embryo (Taylor, 1952) Monkeys develop inapparent infections characterized by moderately sustained viremia and the subsequent development of antibody. The virus multiplies in various cell lines in tissue culture and shows marked cytopathogenicity for HeLa cells and human adult fibroblasts (Moore, 1957) It has physical properties similar to related viruses, including [illegible]

The basis for placing the virus in Group B has been discussed in Chapter 12, and detailed analysis by cross-complement-fixation tests has shown a slightly closer relationship to yellow fever virus than to other members of the group, although this is not supported by HI or neutralization test (Casals, personal communication). The observation that a rise in yellow fever antibodies occurs in a yellow-fever-immune human being or animal following infection with Zika virus (Bearcroft, 1956) is to be expected following superinfection with any Group B virus

Experimental transmission of the virus by *Aëdes aegypti* mosquitoes was demonstrated by Boorman and Porterfield (1956). Neutralization of the virus by the serum of human inhabitants of Uganda and Tanganyika was demonstrated in 6 to 13 per cent of those tested (Dick et al, 1952; Smithburn, 1952) In India, Malaya and Borneo, 17 to 19 per cent neutralization rates were found (Smithburn et al, 1954a, Smithburn, 1954) As in the case of Uganda S, however, there was a strong tendency for the same sera to neutralize other Group B viruses Therefore, such results are difficult to assess.

NTAYA VIRUS

Ntaya virus was isolated by Smithburn and Haddow (1951) from a mixed lot of wild-caught mosquitoes in Uganda So far, a subsequent isolation has not been reported The virus has been shown to multiply in *Aëdes aegypti* mosquitoes infected by inoculation (Whitman, personal communication). It is pathogenic for albino Swiss mice by the intracerebral route and reaches after adaptation a titer of about 10^{-6} in mouse brain. It is not pathogenic for rhesus monkeys and apparently fails to multiply in them, since repeated large inocula are required to produce demonstrable antibodies It produces a fatal infection in chick embryos following inoculation by the yolk and amniotic sac routes (Taylor, 1952) Some degree of virus multiplication has been observed in various cell lines in tissue culture, but cytopathogenicity was marked only for human adult fibroblasts (Moore, 1957) The size as determined by gradocol membrane

filtration has been reported as 80 to 122 mμ (Smithburn and Bugher, 1953), which is much larger than any other member of Group B so far studied A hemagglutinin is demonstrable in suckling mouse brain (Casals and Brown, 1954)

Antibodies neutralizing Ntaya virus have been found in the sera of residents of various parts of the world, and frequently the incidence of such antibodies has been quite high In Egypt, for example, an over-all rate of 34 9 per cent was found (Smithburn et al, 1954b) The extensive virologic studies carried out in Egypt by Taylor and his associates (1956) failed to result in a single isolation of Ntaya virus One striking aspect of all recent surveys has been that Ntaya-positive sera in almost all cases also neutralize at least one other Group B virus; the positive sera in Egypt are associated with West Nile virus neutralization. The capacity to neutralize Ntaya has been demonstrated to develop in convalescent sera from known West Nile infections In other parts of the world, a similar association is found with dengue and Japanese B antibodies where these represent the prevalent infections It seems extremely doubtful, therefore, that Ntaya virus has been demonstrated to infect man, a resonable explanation of the high incidence of neutralization is that the antigenic structure of Ntaya virus is such as to render it particularly susceptible to the action of various Group B antibodies

WESSELSBRON VIRUS INFECTION

Wesselsbron virus was first described by Weiss, Haig and Alexander (1956) who isolated it from a dead lamb in the Orange Free State in South Africa Shortly after the original isolation, two additional strains were obtained in Natal, South Africa, by Smithburn et al (1957), one from human blood and one from mosquitoes

The virus is the cause of abortion and death in pregnant sheep and of death in newborn lambs It is not yet known whether it has a similar effect in other domestic animals, nor has it been determined how serious a cause of economic loss this infection may be The virus causes an influenzalike febrile disease in man in which a rash may be seen and from which convalescence may be somewhat prolonged Knowledge of the human infection is based on 1 naturally and 4 laboratory acquired infections.

Degenerative changes are seen in the liver *of pregnant ewes and lambs dying from the* disease, and virus can be isolated from liver and brain of dead lambs. Infection of pregnant guinea pigs and rabbits also results in abortion or neonatal death of the young Thus, the virus appears to be pantropic with a predilection for embryonic mammalian tissue Early studies showed a low mortality but a high morbidity rate among nonpregnant adult sheep, nonpregnant cattle, horses and pigs experience a mild febrile immunizing infection Nonpregnant guinea pigs, rabbits and *Cercopithecus* monkeys show symptomless infection but may be immunized As with many other Group B viruses, adult mice succumb to infection by the intracerebral route, and suckling mice by both the intracerebral and the intraperitoneal routes The virus attains a brain titer in mice of around $10^{-7.6}$. The embryonated egg is a good host for virus multiplication, but deaths are inconstant and irregular The size of the virus as determined by Polson is approximately 30 mμ (Weiss et al, 1956). Casals (1957) demonstrated a hemagglutinin in suckling mouse brain and classified the virus as a member of Group B (virus designated SA H177 in reference)

The virus has been isolated from naturally infected *Aedes circumluteolus*, *Aedes caballus*, and *Taeniorhynchus uniformis* mosquitoes in South Africa and experimental transmission accomplished with the first 2 species (Muspratt et al, 1957; Smithburn, personal communication). Virus has been isolated from animals or mosquitoes in rather widely separated areas of the Union of South Africa, including the Orange Free State, Northeastern

ease in sheep (Smithburn, personal communication) A high incidence of antibody has been found in domestic animals over a wide area of South Africa and in human beings in Northeastern Natal, the only area so far

studied (Weiss et al., 1956; Smithburn et al, 1957) It is too early to know whether this newly recognized disease occurs in other parts of the world.

BAT SALIVARY GLAND VIRUS

Burns and his associates first reported the isolation of a virus from the salivary glands *of Mexican free-tailed bats* in Texas (Burns and Farinacci, 1956; Burns et al., 1957) An apparently identical virus has been isolated repeatedly from the same species of bat in California (Johnson, unpublished results) The virus is pathogenic for mice by the intracerebral route and also produces a lethal encephalitis when inoculated into Mexican *free-tailed bats by the same route* It is not pathogenic for several other common laboratory animals, including chick embryos, but multiplies in the latter Although virus can be isolated from the brains of intracerebrally inoculated bats, it has not been recovered from the brains, the spleens or the hearts of the animals found infected in nature A hemagglutinin has been demonstrated and the virus has been placed in the arthropod-borne Group B by virtue of its immunologic relationship to St Louis, Japanese B, Murray Valley, West Nile and Ntaya viruses (Burns et al, 1957) It is of interest that it fails to multiply when inoculated into mosquitoes of 3 different genera *Anopheles quadrimaculatus, Culex fatigans* and *Aedes aegypti* (Whitman, personal communication) Its significance is unknown beyond the fact that it represents the second member of Group B demonstrated within the United States.

SPONDWENI VIRUS

Spondweni virus was isolated recently from *Taeniorhynchus uniformis* mosquitoes in northern Natal, South Africa, by Kokernot et al (1957) It is pathogenic for mice by the intracerebral route but not for several other species of laboratory animals A hemagglutinin has been demonstrated and the virus classified by serologic methods as a member of Group B. Preliminary results indicate that antibody capable *of neutralizing the virus can* be detected in the sera of some human residents of an area near that yielding the in*fected mosquitoes.*

REFERENCES

(For complete references, see also second edition, Chapter 11)

Alekseyev, B P, and Gulamova, V P, 1954, Natural immunization of the population in foci of tick-borne encephalitis *in* Smorodintsev, A A, Neurotropic Virus Infections (English Translation), pp 186-189, Medgiz, Leningrad

Anderson, C. R, Aitken, T H G, and Downs, W G, 1956, The isolation of Ilheus virus from wild caught forest mosquitoes in Trinidad, Am J Trop Med & Hyg *5, 621-625.*

Anderson, C R, Aitken, T H G, Downs, W G, and Spence, L, 1957, The isolation of St Louis virus from Trinidad mosquitoes, Am J Trop Med & Hyg *6, 688-692*

Anderson, S G, 1953, Murray valley encephalitis A survey of avian sera, 1951-1952, M J Australia *1, 573-576*

Anderson, S G, Donnelley, M, Stevenson, W J, Caldwell, N J, and Eagle, M, 1952, Murray valley encephalitis Surveys of human and animal sera, M. J Australia *1, 110-114*

Anderson, S G, and Eagle, M, 1953, Murray valley encephalitis The contrasting epidemiological picture in 1951 and 1952, M J Australia *2, 478-481*

Bawell, M B, Deuel, R E, Jr., Matumoto, M, and Sabin, A B, 1950, Status and significance of in*apparent infection with virus of Japanese* B encephalitis in Japan in 1946, Am J Hyg *51, 1-12*

Beadle, L D Menzies, G C, Hayes, G R, Jr, von Zuben, F. J, Jr, and Eads, R B, 1957, St Louis encephalitis in Hidalgo County, Texas Vector evaluation and control Pub Health Rep *72, 531-535*

Bearcroft, W G C, 1956, Zika virus infection experimentally induced in a human volunteer, Tr Roy Soc Trop Med & Hyg *50, 442-448*

Bedjanic, M, Rus, S, Kmet, J, and Vesenjak-Zmijanac, J, 1955, Virus meningo-encephalitis in Slovenia 2 Clinical observations Bull World Health Organ *12, 503-512*

Bernkopf, H, Levine, S, and Nerson, R, 1953, Isolation of West Nile virus in Israel, J Infect Dis *93, 207-218*

Bhatt, P. N, and Work, T W, 1957, Tissue culture studies on arbor viruses of the Japanese B-West Nile complex, Proc Soc Exper Biol & Med *96,* 213-218

Blattner, R J, and Heys, F M, 1944, Blood-sucking vectors of encephalitis Experimental transmission of St Louis encephalitis (Hubbard strain) to white Swiss mice by the American dog tick, *Dermacentor variabilis* Say, J Exper Med *79, 439-454*

Boorman, J P T, and Porterfield, J S, 1956, A simple technique for infection of mosquitoes with viruses Transmission of Zika virus Tr Roy Soc Trop Med & Hyg *50, 238-242*

Buescher, E L, 1956, Arthropod-borne encephalitis in Japan and Southeast Asia, Am J Pub Health *46*, 597-600

Buescher, E L, Chanock, R M, and Philpot, V. B., 1958, Immunology of Japanese encephalitis virus infections I Antibody responses following overt infection in man In preparation

Burnet, F M, 1952, Murray valley encephalitis, Am J Pub Health *42*, 1519-1521

Burns, K F, 1950, Congenital Japanese B encephalitis infection of swine, Proc Soc Exper Biol & Med *75*, 621-625

Burns, K F, and Farinacci, C J, 1956, Virus of bats antigenically related to St Louis encephalitis, Science *123*, 227-228

Burns, K F, Farinacci, C J, and Shelton, D F, 1957, Virus of bats antigenically related to group B arthropod-borne encephalitis viruses, Am J Clin Path *27*, 257-264

Burns, K F, Tigertt, W D, and Matumoto, M, 1949, Japanese equine encephalomyelitis, 1947 epizootic II Serological and etiological studies Am J Hyg *50*, 27-45

Casals, J, 1957, The arthropod borne group of animal viruses, Tr New York Acad Sc Ser 2, *19*, 219-235

Casals, J, and Brown, L V, 1954, Hemagglutination with arthropod-borne viruses, J Exper Med *99*, 429-449

Casals, J., and Webster, L T, 1944, Relationship of the virus of louping ill in sheep and the virus of Russian spring-summer encephalitis in man, J Exper Med *79*, 45-63

Causey, O R, and Theiler, M, 1958, Virus antibody survey on sera of residents of the Amazon valley in Brazil, Am J Trop Med & Hyg *7*, 36-41

Chamberlain, R W, Kissling, R E, Stamm, D D, and Sudia, W D, 1957, Virus of St Louis encephalitis in three species of wild birds, Am J Hyg *65*, 110-118

Chanock, R M, and Sabin, A B, 1953, The hemagglutinin of St Louis encephalitis virus, J Immunol *70*, 271-316.

Chin, T D Y, Heimlich, C R, White, R F, Mason, D M, and Furcolow, M E, 1957, St Louis encephalitis in Hidalgo County, Texas, Epidemiological features Pub Health Rep *72*, 512-518

Chumakov, M P, 1955, Editor, Russian translation of 2nd edition of this volume Moscow, Publishing House of Foreign Literature

Chumakov, M P, Vorobieva, N N, and Sofronova, N E, 1940 The detection of the virus of the tick-borne encephalitis of man in certain rodents of the Ural forests, Arch sc biol *59*, 91 (English summary)

Clarke, D H, and Casals, J, 1955, Improved methods for hemagglutination studies with arthropod-borne viruses, Proc Soc Exper Biol & Med *88*, 96-99

Corristan, E C, La Motte, L C, Jr., and Smith, D G, 1956, Susceptibility of bats to certain encephalitis viruses, Fed Proc *15*, 584

Davidenkov, S N, Kul'kova, Ye F, Pokrovskaya, O A, and Shtulbans, I I, 1954, Clinical aspects of biundulant virus meningoencephalitis *in* Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 38-90, Medgiz, Leningrad

Day, M F, and Bennetts, M J, 1954, A review of problems of specificity in arthropod vectors of plant and animal viruses Commonwealth Scientific and Industrial Research Organization, Canberra, Australia

Deuel, R E, Jr, Bawell, M B, Matumoto, M, and Sabin, A B, 1950, Status and significance of inapparent infection with virus of Japanese B encephalitis in Korea and Okinawa in 1946, Am J Hyg *51*, 13-20

Dick, G W A, 1953, Epidemiological notes on some viruses isolated in Uganda (Yellow fever, Rift Valley fever, Bwamba fever, West Nile, Mengo, Semliki forest, Bunyamwera, Ntaya, Uganda S, and Zika viruses) Tr Roy Soc Trop Med & Hyg *47*, 13-43.

Dick, G W A, and Haddow, A J, 1952, Uganda S virus A hitherto unrecorded virus isolated from mosquitoes in Uganda, Tr Roy Soc Trop Med & Hyg *46*, 600-618

Dick, G W A, Kitchen, S F, and Haddow, A J, 1952, Zika virus, Tr Roy Soc Trop Med & Hyg *46*, 509-514

Dickerson, R B, Newton, J R, and Hansen, J E, 1952, Diagnosis and immediate prognosis of Japanese B encephalitis, Am J Med *12*, 277-288

Downs, W G, Anderson, C R, Aitken, T H G, and Delpeche, K A, 1956a, Notes on the epidemiology of Ilheus virus infection in Trinidad, B W I, Caribbean M J *18*, 74-79

Downs, W G, Anderson, C R, and Casals, J, 1957, The isolation of St Louis virus from a nestling bird in Trinidad, British West Indies, Am J Trop Med & Hyg *6*, 693-696,

Downs, W G, Anderson, C R, and Theiler, M, 1956b, Neutralizing antibodies against certain viruses in the sera of residents of Trinidad, B W I, Am J Trop Med & Hyg *5*, 626-641

Drobyshevskaya, A I, 1954a, The complement fixation test in diagnosing Japanese B encephalitis *in* Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 250-265, Medgiz, Leningrad

———, 1954b, The reservoirs of Japanese B encephalitis virus in human and horse blood in endemic areas during the preepidemic and epidemic periods *in* Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 217-222, Medgiz, Leningrad

Drobyshevskaya, A I, Il'yenko, V I, and Korshunova, V A, 1954, Serological characteristics of the blood of persons who have had biundulant virus meningoencephalitis *in* Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 133-141, Medgiz, Leningrad

Drobyshevskaya, A I, and Sokolov, N N, 1954, Serological properties of the blood of domestic animals and birds in areas where Japanese B encephalitis is endemic *in* Smorodintsev, A A., Neurotropic Virus Infections (English translation), pp 298-305, Medgiz, Leningrad

studied (Weiss et al., 1956; Smithburn et al., 1957). It is too early to know whether this newly recognized disease occurs in other parts of the world

BAT SALIVARY GLAND VIRUS

Burns and his associates first reported the isolation of a virus from the salivary glands of Mexican free-tailed bats in Texas (Burns and Farinacci, 1956, Burns et al, 1957). An apparently identical virus has been isolated repeatedly from the same species of bat in California (Johnson, unpublished results) The virus is pathogenic for mice by the intracerebral route and also produces a lethal encephalitis when inoculated into Mexican free-tailed bats by the same route. It is not pathogenic for several other common laboratory animals, including chick embryos, but multiplies in the latter Although virus can be isolated from the brains of intracerebrally inoculated bats, it has not been recovered from the brains, the spleens or the hearts of the animals found infected in nature A hemagglutinin has been demonstrated and the virus has been placed in the arthropod-borne Group B by virtue of its immunologic relationship to St Louis, Japanese B, Murray Valley, West Nile and Ntaya viruses (Burns et al, 1957) It is of interest that it fails to multiply when inoculated into mosquitoes of 3 different genera *Anopheles quadrimaculatus*, *Culex fatigans* and *Aedes aegypti* (Whitman, personal communication) Its significance is unknown beyond the fact that it represents the second member of Group B demonstrated within the United States.

SPONDWENI VIRUS

Spondweni virus was isolated recently from *Taeniorhynchus uniformis* mosquitoes in northern Natal, South Africa, by Kokernot et al. (1957) It is pathogenic for mice by the intracerebral route but not for several other species of laboratory animals. A hemagglutinin has been demonstrated and the virus classified by serologic methods as a member of Group B. Preliminary results indicate that antibody capable of neutralizing the virus can be detected in the sera of some human residents of an area near that yielding the infected mosquitoes.

REFERENCES

(For complete references, see also second edition, Chapter 11)

Alekseyev, B P, and Gulamova, V P., 1954, Natural immunization of the population in foci of tick-borne encephalitis *in* Smorodintsev, A A, Neurotropic Virus Infections (English Translation), pp 186-189, Medgiz, Leningrad

Anderson, C R, Aitken, T H G, and Downs, W G, 1956, The isolation of Ilheus virus from wild caught forest mosquitoes in Trinidad, Am J Trop Med & Hyg. *5*, 621-625

Anderson, C R, Aitken, T H G, Downs, W, G, and Spence, L, 1957, The isolation of St Louis virus from Trinidad mosquitoes, Am J Trop Med & Hyg *6*, 688-692

Anderson, S G, 1953, *Murray valley encephalitis A survey of avian sera, 1951-1952*, M J Australia *1*, 573-576

Anderson, S G, Donnelley, M, Stevenson, W J, Caldwell, N J, and Eagle, M, 1952, Murray valley encephalitis Surveys of human and animal sera, M J Australia *1*, 110-114

Anderson, S G, and Eagle, M, 1953, Murray valley encephalitis The contrasting epidemiological picture in 1951 and 1952, M J Australia *1*, 478-481

Bawell, M B, Deuel, R E, Jr, Matumoto, M., and Sabin, A B, 1950, Status and significance of inapparent infection with virus of Japanese B encephalitis in Japan in 1946, Am J Hyg *51*, 1-12

Beadle, L D, Menzies, G C, Hayes, G R, Jr, von Zuben, F J, Jr, and Eads, R B., 1957, St Louis encephalitis in Hidalgo County, Texas Vector evaluation and control *Pub Health Rep 72*, 531-535

Bearcroft, W G C, 1956, Zika virus infection experimentally induced in a human volunteer, Tr Roy Soc Trop Med & Hyg *50*, 442-448

Bedjanic, M, Rus, S, Kmet, J, and Vesenjak-Zmijanac, J, 1955, Virus meningo-encephalitis in Slovenia 2 Clinical observations Bull World Health Organ *12*, 503-512

Bernkopf, H, Levine, S, and Nerson, R, 1953, Isolation of West Nile virus in Israel, J Infect Dis *93*, 207-218

Bhatt, P N, and Work, T W., 1957, Tissue culture studies on arbor viruses of the Japanese B-West Nile complex, Proc Soc Exper Biol & Med *96*, 213-218

Blattner, R J, and Heys, F M, 1944, Blood-sucking vectors of encephalitis Experimental transmission of St Louis encephalitis (Hubbard strain) to white Swiss mice by the American dog tick, *Dermacentor variabilis* Say, J Exper Med *79*, 439-454

Boorman, J P T., and Porterfield, J S, 1956, A simple technique for infection of mosquitoes with viruses Transmission of Zika virus Tr Roy Soc Trop Med & Hyg *50*, 238-242

Konowalchuk, J, Russ, S B, and Buescher, E L, 1957, Russian spring-summer encephalitis-like (RSSE) viruses from European Russia and Southeast Asia, Bact Proc 57, 66

Koprowska, I, and Koprowski, H, 1953, Morphologic and biologic changes in a mouse ascites tumor following induced infection with certain viruses, Cancer Res 13, 651-660

Koprowski, H, 1956, Ascites tumors as culture media in quantitative growth studies of viral agents, Ann New York Acad Sc 63, 895-914

Koprowski, H, and Hughes, T. P., 1946, The virus of Ilheus encephalitis Physical properties, pathogenicity and cultivation, J Immunol 54, 371-385

Koprowski, H, and Norton, T W, 1950, Interference between certain neurotropic viruses and transplantable mouse tumors, Cancer 3, 874-885

Krejci, J, 1949, Isolement d'un virus nouveau au cours d'une épidémie de méningo-encéphalite dans la région de Vyskov (Moravie), Presse med 57, 1084.

Kulkarni, K G, Work, T H, Clarke, D H, and Casals, J, 1958, Kyasanur Forest Disease V Serological Studies with Kyasanur Forest Disease Virus To be published

Kunin, C M, and Chin, T D Y, 1957, St Louis encephalitis in Hidalgo County, Texas Clinical and pathological features Pub Health Rep 72, 519-525

Laemmert, H W., Jr., and Hughes, T P., 1947, The virus of Ilheus encephalitis Isolation, serological specificity and transmission J Immunol 55, 61-67

Lawson, J H, Manderson, W G, and Hurst, E W, 1949, Louping ill meningo-encephalitis A further case and a serological survey Lancet 257, 696-699

Lennette, E H., and Koprowski, H., 1944, Neutralization tests with certain neurotropic viruses A comparison of the sensitivity of the extraneural and intracerebral routes of inoculation for the detection of antibodies J Immunol 49, 375-385

Lennette, E H, and Longshore, W A, Jr, 1951, Western equine and St Louis encephalitis in man, California, 1945 1950, California Med 75, 189-195

Levkovich, E N., Sarmanova, E S, and Dumina, A L, 1955, Experimental study of the role of animals in the dissemination of the virus of tick-borne (spring-summer) encephalitis in natural foci Medgiz, Leningrad, pp 283-288 (see Vet Bull 27, 349, 1957)

Likar, M, and Kmet, J, 1956, Virus meningo-encephalitis in Slovenia 4 Isolation of the virus from the ticks "Ixodes ricinus" Bull World Health Organ 15, 275-279.

Lincoln, A F, and Sivertson, S E, 1952, Acute phase of Japanese B encephalitis Two hundred and one cases in American soldiers, Korea, 1950 J A M A 150, 268-273

Longshore, W A, Jr, 1955, Some clinical aspects of western equine and St Louis encephalitis Proc and Papers of Calif Mosq Control Assoc, 23rd annual conf

Longshore, W A, Jr, Stevens, I M, Hollister, A C, Jr, Gittelsohn, A, and Lennette, E H, 1956, Epidemiologic observations on acute infectious encephalitis in California with special reference to the 1952 outbreak, Am J Hyg 63, 69-86

McCollum, R W, and Foley, J F, 1957, Japanese B encephalitis virus in tissue culture, Proc Soc Exper Biol & Med 94, 556-560

MacDonald, F., 1952, Haemagglutination with the virus of Murray valley encephalitis (MVE), Brit J Exper Path, 33, 537-542.

McLean, D M, 1953a, The behaviour of Murray valley encephalitis virus in young chickens, Australian J. Exper Biol & M Sc 31, 491-503

———, 1953b, Transmission of Murray valley encephalitis virus by mosquitoes, Australian J. Exper Biol & M Sc 31, 481-490

———, 1956, Serological group reactions of Murray valley encephalitis virus, Australian J Exper. Biol & M Sc 34, 71-75.

———, 1957, Vectors of Murray valley encephalitis, J Infect Dis 100, 223-227

McLean, D. M., and Stevenson, W J, 1954, Serological studies on the relationship between Australian X-disease and the virus of Murray valley encephalitis, M J Australia 1, 636-638

MacNamara, F N, 1953a, Reactions following neurotropic yellow fever vaccine given by scarification in Nigeria, Tr Roy Soc. Trop Med & Hyg 47, 199-208

———, 1953b, Uganda S and yellow fever viruses A slight relationship shown by experiments in rhesus monkeys and white mice Brit J Exper Path 34, 392-399

———, 1954, Zika virus A report on three cases of human infection during an epidemic of jaundice in Nigeria, Tr Roy Soc Trop Med & Hyg 48, 139-145

Marburg, K., Goldblum, N, Sterk, V V, Jasinska-Klingberg, W, and Klingberg, M A., 1956, The natural history of West Nile fever I Clinical observations during an epidemic in Israel. Am J Hyg 64, 259-269

Melnick, J L, Paul, J R, Riordan, J T., Barnett, V H, Goldblum, N, and Zabin, E, 1951, Isolation from human sera in Egypt of a virus apparently identical to West Nile virus, Proc Soc Exper Biol & Med 77, 661-665

Miles, J A R, 1952, An encephalitis virus isolated in south Australia I Some characteristics of the virus Australian J Exper Biol & M Sc 30, 341-351

———, 1955, A note on the persistence and distribution of antibodies to Murray valley encephalitis in man in south Australia, M J Australia 1, 1-2

Miles, J A R, and Dane, D M S, 1956, Further observations relating to Murray valley encephalitis in the Northern Territory of Australia, M J Australia 1, 389-393

Miles, J A R, Fowler, M C, and Howes, D W., 1951, Isolation of a virus from encephalitis in south Australia A preliminary report M J Australia 1, 799-800

Miles, J A R, and Howes, D W, 1953, Observations on virus encephalitis in south Australia, M J Australia 1, 7-12

Edward, D G, 1948, *Immunization against louping ill Immunization of man* Brit J. Exper Path *29, 372-378*

———, 1950, The relationship of a newly-isolated human encephalitis virus to louping ill virus, Brit J Exper Path *31, 515-522*

Federation of Malaya, 1957, Annual Report of the Institute for Medical Research for 1955, Kuala Lumpur

Fendrich, J, Nir, Y, and Goldwasser, R, 1957, Studies on the behavior of West Nile and western equine encephalomyelitis viruses in embryonated eggs, J Infect Dis *100*, 141-146.

Finley, K H., and Hollister, A C, 1951, Western equine and St Louis encephalomyelitis, distribution and histological nature of central nervous system lesions, California Med *74, 225-229*

Fornosi, F, and Molnar, E, 1954, Zeckenencephalitis in Ungarn, Acta Microbiologica. Budapest, *1*, 21 (German summary)

French, E L, 1952, Murray valley encephalitis Isolation and characterization of the aetiological agent, M J Australia *1*, 100-103

French, E L, Anderson, S G, Price, A V G, and Rhodes, F. A, 1957, Murray Valley encephalitis in New Guinea *I* Isolation of Murray valley encephalitis virus from the brain of a fatal case of encephalitis occurring in a Papuan native Am J Trop Med & Hyg *6, 827-834*

Gagarina, A V, and Netskii, G I, 1955, Materials relating to the spread and the vectors of haemorrhagic fever in western Siberia, Medgiz, Leningrad, pp. *220-224* (see *Rev Appl Ent*, *44*, Ser B, 150-151, 1956)

Gajdusek, D C, 1953, Acute Infectious Hemorrhagic Fevers and Mycotoxicoses in the Union of Soviet Socialist Republics Med Sci Pub No. 2, Army Medical Service Graduate School, Washington, D C, pp. 19-35

Garven, A K, and Margolis, J, 1952, A fatal case of Murray valley encephalitis occurring at Narrabri in New South Wales, M J Australia *2, 621-624*

Goldblum, N, Sterk, V V, and Jasinska-Klingberg, W, 1957, The natural history of West Nile fever II Virological findings and the development of homologous and heterologous antibodies in West Nile infection in man, Am J. Hyg *66, 363-380*

Goldblum, N, Sterk, V V, and Paderski, B, 1954, West Nile fever The clinical features of the disease and the isolation of West Nile virus from the blood of nine human cases Am J Hyg *59, 89-103*

Grinschgl, G, 1955, Virus meningo-encephalitis in Austria *2* Clinical features, pathology and diagnosis, Bull World Health Organ *12, 535-564*

Hale, J H, Colless, D H, and Lim, K A, 1957, Investigation of the Malaysian form of *Culex tritaeniorhynchus* as a potential vector of Japanese B encephalitis virus on Singapore island, Ann Trop Med *51, 17-25*

Hale, J H, and Lee, L H, 1954, Transplacental passage of antibody to Japanese B encephalitis virus, J. Path. & Bact *68*, 631-632.

Hammon, W M, Rees, D M, Casals, J., and Meiklejohn, G, 1949, Experimental transmission of Japanese B encephalitis virus by *Culex tritaeniorhynchus* and *Culex pipiens* var *pallens*, suspected natural vectors, Am. J Hyg *50*, *46-50*

Hammon, W M, and Reeves, W C, 1943, Laboratory transmission of St. Louis encephalitis virus by three genera of mosquitoes, *J* Exper Med *78*, 241-253

Hammon, W M., Reeves, W. C, and Sather, G E, 1951a, Western equine and St Louis encephalitis viruses in the blood of experimentally infected wild birds and epidemiological implications of findings, J. Immunol *67, 357-367.*

———, 1951b, Japanese B encephalitis virus in the blood of experimentally inoculated birds (Epidemiological implications) Am J. Hyg *53*, 249-261

Hammon, W M, and Sather, G E, 1956, Immunity of hamsters to West Nile and Murray valley viruses following immunization with St Louis and Japanese B Proc Soc Exper Biol & Med *91*, 521-524

Hammon, W M, Tigertt, W D, Sather, G E, and Schenker, H, 1949, Isolation of Japanese B encephalitis virus from naturally infected *Culex tritaeniorhynchus* collected in Japan, Am J Hyg *50, 51-56*

Hloucal, L, and Gallia, F, 1949, An epidemic of neurotropic virus disease in the district of Strakonice 1948 Sbornik Lekarsky, *51*, 374-375 (English Summary).

Hullinghorst, R L, Burns, K F, Choi, Y T, and Whatley, L. R, 1951, Japanese B encephalitis in Korea The epidemic of 1949 J A M A *145*, 460-466

Hurlbut, H S, 1950, The transmission of Japanese B encephalitis by mosquitoes after experimental hibernation, Am J Hyg *51, 265-268*

———, 1956, West Nile virus infection in arthropods, Am J Trop. Med & Hyg *5, 76-85*

Jettmar, H M, 1956, Tick-borne virus encephalitis in Austria [illegible] Jagdwissensch, *2*, 174-180 (See Bull Hyg 32, 216, 1957)

Kerr, J A, 1952, Studies on certain viruses isolated in the tropics of Africa and South America Immunological reactions as determined by cross complement-fixation tests J Immunol *68*, 461-472

Kmet, J, Vesenjak-Zmijanac, J, Bedjanic, M, and Rus, S, 1955, Virus meningoencephalitis in Slovenia *1* Epidemiological observations, Bull World Health Organ *12*, 491-501.

Kokernot, R H, Shinefield, H R, and Longshore, W A., Jr., 1953, The 1952 outbreak of encephalitis in California Differential diagnosis, California Med *79, 73-77*

Kokernot, R H, Smithburn, K C, Muspratt, J, and Hodgson, [illegible], 1957, Studies on arthropod borne viruses of Tongaland, VIII Spondweni virus, an agent previously unknown, isolated from *Taeniorhynchus (Mansonioides) uniformis* Theo., South African J M Sc *22*, 103-112

Kokernot, R H, Smithburn, K C, and Weinbren, M P., 1956, Neutralizing antibodies to arthropod-borne viruses in human beings and animals in the Union of South Africa, J. Immunol. *77*, 313-323

cephalitis virus hemagglutinin, Proc Soc Exper Biol & Med 74, 222-230

Sabin, A B, Duffy, C E, Warren, J, Ward, R, Peck, J L, Jr, and Ruchman, I, 1943, The St Louis and Japanese B types of epidemic encephalitis Development of noninfective vaccines. Report of basic data J A M A 122, 477-486

Sabin, A B, Ginder, D R, and Matumoto, M, 1947, Difference in dissemination of the virus of Japanese B encephalitis among domestic animals and human beings in Japan, Am J Hyg 46, 341-355

Scherer, W F, and Syverton, J T, 1954, The viral range in vitro of a malignant human epithelial cell (strain HeLa, Gey) II Studies with encephalitis viruses of the eastern, western, West Nile, St Louis and Japanese B types. Am J Path 30, 1075-1084

Shapoval, A N, Dankovskiy, N L, Drobyshevskaya, A I, and Sokolov, N N, 1954, Clinical aspects and immunology of attenuated forms of Japanese B encephalitis in Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 305-313, Medgiz, Leningrad

Sharpless, G R, Davies, M C, and Cox, H R, 1950, Antagonistic action of certain neurotropic viruses toward a lymphoid tumor in chickens with resulting immunity, Proc Soc Exper Biol & Med 73, 270-275

Shimizu, T, Kawakimi, Y, Fukuhara, S, and Matumoto, M, 1954, Experimental still birth in pregnant swine infected with Japanese encephalitis virus, Jap J Exper Med 24, 363-375

Silber, L A, and Soloviev, V D, 1946, Far Eastern tick-borne spring-summer (spring) encephalitis, Am Review Soviet Med, special supplement, pp 6-80

Smadel, J E, Randall, R, and Warren, J, 1947, Preparation of Japanese encephalitis vaccine Bull U S Army Med Dept 7, 963-972

Smith, C E G, 1956, A virus resembling Russian spring summer encephalitis virus from an Ixodid tick in Malaya, Nature, London 178, 581-582

———, 1957, A localized outbreak of dengue fever in Kuala Lumpur Serological aspects J Hyg 55, 207-223

Smith, D G, and Herring, A S, 1956, Cultivation and titration of Japanese B encephalitis virus in embryonated eggs, Fed Proc 15, 614

Smith, M G, 1943, Placental transmission of immunity to St Louis encephalitis virus inoculated intraperitoneally in mice, Proc Soc Exper Biol & Med 52, 83-85

Smithburn, K C, 1952, Neutralizing antibodies against certain recently isolated viruses in the sera of human beings residing in East Africa, J. Immunol 69, 223-234

———, 1954, Neutralizing antibodies against arthropod-borne viruses in the sera of long-time residents of Malaya and Borneo, Am J Hyg 59, 157-163

Smithburn, K C, and Bugher, J C, 1953, Ultrafiltration of recently isolated neurotropic viruses, J Bact 66, 173-177.

Smithburn, K C, and Haddow, A J, 1951, Ntaya virus A hitherto unknown agent isolated from mosquitoes collected in Uganda, Proc Soc Exper Biol & Med 77, 130-133

Smithburn, K C, and Jacobs, H R, 1942, Neutralization-tests against neurotropic viruses with sera collected in Central Africa, J Immunol 44, 9-23

Smithburn, K C, Kerr, J A, and Gatne, P B, 1954a, Neutralizing antibodies against certain viruses in the sera of residents of India, J Immunol 72, 248-257

Smithburn, K C, Kokernot, R H, Weinbren, M P, and de Meillon, B, 1957, Studies on arthropod-borne viruses of Tongaland IX Isolation of Wesselsbron virus from a naturally infected human being and from *Aëdes* (*Banksinella*) *circumluteolus* Theo mosquitoes, South African J M Sc 22, 113-120.

Smithburn, K C, Taylor, R M, Rizk, F, and Kader, A, 1954b, Immunity to certain arthropod-borne viruses among indigenous residents of Egypt Am J Trop Med & Hyg 3, 9-18

Smorodintsev, A A, Drobyshevskaya, A I, Il'yenko, V I, Alekseyev, B P, Gulamova, V P, and Fedorchuk, L V, 1954a, Etiology and epidemiology of a new neurotropic virus infection—biundulant meningoencephalitis in Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 4-37, Medgiz, Leningrad

Smorodintsev, A A, Yablonovskaya, L Ya, and Bibikov, D I, 1954b, Recovery of virus and detection of antibodies of Japanese B encephalitis in wild birds in an endemic area in Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 266-281, Medgiz, Leningrad

South African Institute for Medical Research, Annual Report for 1956, Johannesburg, pp 53-56

Southam, C M, 1956a, Serologic studies of encephalitis in Japan I Hemagglutination-inhibiting, complement-fixing and neutralizing antibody following overt Japanese B encephalitis J Infect Dis 99, 155-162

———, 1956b, Serologic studies of encephalitis in Japan II Inapparent infections by Japanese B encephalitis virus J Infect Dis 99, 163-169

Southam, C M, and Moore, A E, 1951, West Nile, Ilheus and Bunyamwera virus infections in man, Am J Trop Med 31, 724-741

———, 1952, Clinical studies of viruses as antineoplastic agents, with particular reference to Egypt 101 virus, Cancer 5, 1025-1034

———, 1954a, Induced virus infections in man by the Egypt isolates of West Nile virus, Am J Trop Med & Hyg 3, 19 50

———, 1954b, Anti-virus antibody studies following induced infection of man with West Nile, Ilheus and other viruses, J Immunol 72, 446-462

Spence, L P, and Thomas, L, 1958, Application of hemagglutination and complement-fixation techniques to the identification and serological classification of arthropod-borne viruses Studies on Chikungunya and Makonde viruses (in preparation)

Moore, A E, 1954, Effects of viruses on tumors, Ann Rev Microbiol *8, 393-410*

———, 1957, Oncolytic properties of viruses, Texas *Rep Biol & Med 15, 588-602*

Morris, J A, O'Connor, J R, and Smadel, J E, 1955, Infection and immunity patterns in monkeys injected with viruses of Russian spring-summer and Japanese encephalitis, Am J Hyg *62*, 327-341

Muckenfuss, R S, Smadel, J E, and Moore, E, 1938, The neutralization of encephalitis virus (St Louis, 1933) by serum J Clin Invest *17*, 53-57

Muspratt, J, Smithburn, K C, Paterson, H E, and Kokernot, R H, 1957, Studies on arthropod-borne viruses of Tongaland X The laboratory transmission of Wesselsbron virus by the bite of *Aëdes (Banksinella) circumluteolus* Theo, South African J M Sc *22*, 121-126

Oker-Blom, N, 1956, Propagation of louping ill virus in malignant human epithelial cells, strain HeLa, Ann med exper et biol Fenniae *34*, 199-205

Pattyn, S R, and Wyler, R, 1955, Meningo-encephalite a virus en Autriche 4 La viremie dans l'infection experimentale Essais de transmission par les moustiques, Bull World Health Organ *12*, 581-589

Pollard, M, and Bussell, R H, 1952, Oncolytic effect of viruses in tissue cultures, Proc Soc Exper Biol & Med *80*, 574-578

Pond, W L, and Russ, S B, 1955, Serological aspects of virus meningo-encephalitis A study of the reactions of two viruses isolated during the 1953 epidemics in Slovenia and Austria, Bull World Health Organ *12*, 591-594

Pond, W L, Russ, S B, Lancaster, W E, Audy, J R, and Smadel, J E, 1954, Japanese encephalitis in *Malaya II Distribution of neutralizing* antibodies in man and animals Am J Hyg *59*, 17-25

Pond, W L, Russ, S B, Rogers, N G, and Smadel, J E, 1955, Murray valley encephalitis virus Its serological relationship to the Japanese-West Nile-St Louis encephalitis group of viruses, J Immunol *75*, 78-84

Pond, W L, Russ, S B, and Warren, J, 1953, The Russian spring-summer encephalitis and louping ill group of viruses Relationship of European and Asiatic strains of Russian spring-summer encephalitis viruses and louping ill virus J Infect Dis *93*, 294-300

Pond, W L, and Smadel, J E, 1954, Neurotropic viral diseases in the Far East during the Korean war *in* Recent Advances in Medicine and Surgery, vol 2, pp 219-233, Med Sci Pub 4, Army Med Serv Grad School, Washington, D C

Povlovsky, E N, 1941, Vectors, reservoirs and focal distribution of tick encephalitis, Neuropath & Psychiat, Moscow, *10*, 10-12 (see Bull Hyg *17*, 694-695, 1942)

Przesmycki, F., et al, 1956, L'encéphalite a tiques en Pologne, Ann Inst Pasteur *91* (Suppl Dec) 3-8

Rampas, J., and Gallia, F, 1949, Isolace viru encefalitidy z klíšťat Ixodes ricinus, Časop lék česk *88*, 1179 (English summary)

Ranzenhofer, E R, Alexander, E R, Beadle, L D, Bernstein, A, and Pickard, R C, 1957, St Louis encephalitis in Calvert City, Kentucky, 1955 An epidemiologic study, Am J Hyg *65*, 147-161

Reeves, W C, 1951, The encephalitis problem in the United States, Am J Pub Health *41*, 678-686

———, 1953, The knowns and the unknowns in the natural history of encephalitis Proc. and Papers 21st. Annual Conference of the California Mosquito Control Association

Reeves, W C, and Hammon, W. M, 1946, Laboratory transmission of Japanese B encephalitis virus by seven species (three genera) of North American mosquitoes J Exper Med *83*, 185-194

Reeves, W C, Hammon, W M, Doetschman, W H, McClure, H E, and Sather, G, 1955, Studies on mites as vectors of western equine and St Louis encephalitis viruses in California, Am J Trop Med & Hyg *4*, 90-105

Reeves, W C, Hammon, W M, Lazarus, A S, Brockman, B, McClure, H E, and Doetschman, W H, 1952, The changing picture of encephalitis in the Yakima Valley, Washington, J Infect Dis *90*, 291-301

Re [illegible] and

Dis *95*, 168-178

Richling, E, 1955, Virus meningo encephalitis in Austria 1 Epidemiological features, Bull World Health Organ *12*, 521-534

Robertson, E G, 1952, Murray valley encephalitis Pathological aspects M J Australia *1*, 107-110

Robertson, E G, and McLorinan, H, 1952, Murray valley encephalitis Clinical aspects, M J Australia *1*, 103-107

de Rodaniche, E, 1956, Isolation of the virus of Ilheus encephalitis from mosquitoes of the genus *Psorophora* captured in Honduras, Am J Trop Med & Hyg *5*, 797-801

de Rodaniche, E, and Galindo, P, 1957, Isolation of Ilheus virus from *Sabethes chloropterus* captured in Guatemala in 1956, Am J Trop Med & Hyg *6*, 686-687

van Rooyen, C E, and Rhodes, A J, 1948, Virus Diseases of Man, New York, Thomas Nelson & Sons

Ross, R. W, 1956, The Newala epidemic III The virus *Isolation, pathogenic properties and* relationship to the epidemic, J Hyg *54*, 177-191

Rugevich, A S, Ivanova, M G, and Semenova, A N, 1954, Clinical aspects and diagnosis of bi-undulant virus meningoencephalitis *in* Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 91-101, Medgiz, Leningrad

Sabin, A B, 1947, Epidemic encephalitis in military personnel Isolation of Japanese B virus on Okinawa in 1945, serologic diagnosis, clinical manifestations, epidemiologic aspects and use of mouse-brain vaccine J A M A *133*, 281-293

Sabin, A B, and Buescher, E L, 1950, Unique physico-chemical properties of Japanese B en-

cephalitis virus hemagglutinin, Proc Soc Exper Biol & Med 74, 222-230

Sabin, A B, Duffy, C E, Warren, J, Ward, R, Peck, J L, Jr, and Ruchman, I, 1943, The St Louis and Japanese B types of epidemic encephalitis Development of noninfective vaccines Report of basic data J A M A 122, 477-486

Sabin, A B, Ginder, D R, and Matumoto, M, 1947, Difference in dissemination of the virus of Japanese B encephalitis among domestic animals and human beings in Japan, Am J Hyg 46, 341-355

Scherer, W F, and Syverton, J T, 1954, The viral range in vitro of a malignant human epithelial cell (strain HeLa, Gey) II Studies with encephalitis viruses of the eastern, western, West Nile, St Louis and Japanese B types Am J Path 30, 1075-1084

Shapoval, A N, Dankovskiy, N L, Drobyshevskaya, A I, and Sokolov, N N, 1954, Clinical aspects and immunology of attentuated forms of Japanese B encephalitis in Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 305-313, Medgiz, Leningrad

Sharpless, G R, Davies, M C, and Cox, H R, 1950, Antagonistic action of certain neurotropic viruses toward a lymphoid tumor in chickens with resulting immunity, Proc Soc Exper Biol & Med 73, 270-275

Shimizu, T, Kawakimi, Y, Fukubara, S, and Matumoto, M, 1954, Experimental still birth in pregnant swine infected with Japanese encephalitis virus, Jap J Exper Med 24, 363-375

Silber, L A, and Soloviev, V D, 1946, Far Eastern tick-borne spring-summer (spring) encephalitis, Am Review Soviet Med, special supplement, pp 6-80

Smadel, J E, Randall, R, and Warren, J, 1947, Preparation of Japanese encephalitis vaccine Bull U S Army Med Dept 7, 963-972

Smith, C E G, 1956, A virus resembling Russian spring-summer encephalitis virus from an Ixodid tick in Malaya, Nature, London 178, 581-582

———, 1957, A localized outbreak of dengue fever in Kuala Lumpur Serological aspects J Hyg 55, 207-223

Smith, D G, and Herring, A S, 1956, Cultivation and titration of Japanese B encephalitis virus in embryonated eggs, Fed Proc 15, 614

Smith, M G, 1943, Placental transmission of immunity to St Louis encephalitis virus inoculated intraperitoneally in mice, Proc Soc Exper Biol & Med 52, 83-85

Smithburn, K C, 1952, Neutralizing antibodies against certain recently isolated viruses in the sera of human beings residing in East Africa, J Immunol 69, 223-234

———, 1954, Neutralizing antibodies against arthropod-borne viruses in the sera of long time residents of Malaya and Borneo, Am J Hyg 59, 157-163

Smithburn, K C, and Bugher, J C, 1953, Ultrafiltration of recently isolated neurotropic viruses, J Bact 66, 173-177

Smithburn, K C, and Haddow, A J, 1951, Ntaya virus A hitherto unknown agent isolated from mosquitoes collected in Uganda, Proc Soc Exper Biol & Med 77, 130-133

Smithburn, K C, and Jacobs, H R, 1942, Neutralization-tests against neurotropic viruses with sera collected in Central Africa, J Immunol 44, 9 23

Smithburn, K C, Kerr, J A, and Gatne, P B, 1954a, Neutralizing antibodies against certain viruses in the sera of residents of India, J Immunol 72, 248-257

Smithburn, K C, Kokernot, R H, Weinbren, M P, and de Meillon, B, 1957, Studies on arthropod-borne viruses of Tongaland IX Isolation of Wesselsbron virus from a naturally infected human being and from *Aëdes (Banksinella) circumluteolus* Theo mosquitoes, South African J M Sc 22, 113-120

Smithburn, K C, Taylor, R M, Rizk, F, and Kader, A, 1954b, Immunity to certain arthropod-borne viruses among indigenous residents of Egypt Am J Trop Med & Hyg 3, 9-18

Smorodintsev, A A, Drobyshevskaya, A I, Il'yenko, V I, Alekseyev, B P, Gulamova, V P, and Fedorchuk, L V, 1954a, Etiology and epidemiology of a new neurotropic virus infection—biundulant meningoencephalitis in Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 4-37, Medgiz, Leningrad

Smorodintsev, A A, Yablonovskaya, L Ya, and Bibikov, D I, 1954b, Recovery of virus and detection of antibodies of Japanese B encephalitis in wild birds in an endemic area in Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 266-281, Medgiz, Leningrad

South African Institute for Medical Research, Annual Report for 1956, Johannesburg, pp 53-56

Southam, C M, 1956a, Serologic studies of encephalitis in Japan I Hemagglutination-inhibiting, complement-fixing and neutralizing antibody following overt Japanese B encephalitis J Infect Dis 99, 155-162

———, 1956b, Serologic studies of encephalitis in Japan II Inapparent infections by Japanese B encephalitis virus J Infect Dis 99, 163-169

Southam, C M, and Moore, A E, 1951, West Nile, Ilheus and Bunyamwera virus infections in man, Am J Trop Med 31, 724-741

———, 1952, Clinical studies of viruses as antineoplastic agents, with particular reference to Egypt 101 virus, Cancer 5, 1025-1034

———, 1954a, Induced virus infections in man by the Egypt isolates of West Nile virus, Am J Trop Med & Hyg 3, 19 50

———, 1954b, Anti-virus antibody studies following induced infection of man with West Nile, Ilheus and other viruses, J Immunol 72, 446-462

Spence, L P, and Thomas, L, 1958, Application of hemagglutination and complement-fixation techniques to the identification and serological classification of arthropod-borne viruses Studies on Chikungunya and Makonde viruses (in preparation)

Moore, A E, 1954, Effects of viruses on tumors, Ann Rev Microbiol *8*, 393-410

———, 1957, Oncolytic properties of viruses, Texas Rep Biol & Med *15*, 588-602

Morris, J A, O'Connor, J R, and Smadel, J E, 1955, Infection and immunity patterns in monkeys injected with viruses of Russian spring-summer and Japanese encephalitis, Am J Hyg *62*, 327-341

Muckenfuss, R S, Smadel, J E, and Moore, E, 1938, The neutralization of encephalitis virus (St Louis, 1933) by serum J Clin Invest *17*, 53-57

Muspratt, J, Smithburn, K C, Paterson, H E, and Kokernot, R H, 1957, Studies on arthropod-borne viruses of Tongaland X The laboratory transmission of Wesselsbron virus by the bite of *Aedes (Banksinella) circumluteolus* Theo, South African J M Sc *22*, 121-126

Oker-Blom, N, 1956, Propagation of louping ill virus in malignant human epithelial cells, strain HeLa, Ann med exper et biol Fenniae *34*, 199-205

Pattyn, S R, and Wyler, R, 1955, Méningo-encephalite a virus en Autriche 4 La viremie dans l'infection experimentale Essais de transmission par les moustiques, Bull World Health Organ *12*, 581-589

Pollard, M, and Bussell, R H, 1952, Oncolytic effect of viruses in tissue cultures, Proc Soc Exper Biol & Med *80*, 574-578

Pond, W L, and Russ, S B, 1955, Serological aspects of virus meningo-encephalitis A study of the reactions of two viruses isolated during the 1953 epidemics in Slovenia and Austria, Bull World Health Organ *12*, 591-594

Pond, W L, Russ, S B, Lancaster, W E, Audy, J R, and Smadel, J E, 1954, Japanese encephalitis in Malaya II Distribution of neutralizing antibodies in man and animals Am J Hyg *59*, 17-25

Pond, W L, Russ, S B, Rogers, N G, and Smadel, J E, 1955, Murray valley encephalitis virus Its serological relationship to the Japanese-West Nile-St Louis encephalitis group of viruses, J Immunol *75*, 78-84

Pond, W L, Russ, S B, and Warren, J, 1953, The Russian spring-summer encephalitis and louping ill group of viruses Relationship of European and Asiatic strains of Russian spring-summer encephalitis viruses and louping ill virus J Infect Dis *93*, 294-300

Pond, W L, and Smadel, J E, 1954, Neurotropic viral diseases in the Far East during the Korean war *in* Recent Advances in Medicine and Surgery, vol *2*, pp 219-233, Med Sci Pub *4*, Army Med Serv Grad School, Washington, D C

Povlovsky, E N, 1941, Vectors, reservoirs and focal distribution of tick encephalitis, Neuropath & Psychiat., Moscow, *10*, 10-12 (see Bull Hyg *17*, 694-695, 1942)

Przesmycki, F, et al, 1956, L'encéphalite a tiques en Pologne, Ann Inst Pasteur *91* (Suppl Dec) 3-8

Rampas, J., and Gallia, F, 1949, Isolace viru encefalitidy z klíštat Ixodes ricinus, Časop lék česk *88*, 1179 (English summary)

Ranzenhofer, E R, Alexander, E R Beadle, L D, Bernstein, A, and Pickard, R C, 1957, St Louis encephalitis in Calvert City, Kentucky, 1955 An epidemiologic study. Am J. Hyg *65*, 147-161

Reeves, W. C, 1951, The encephalitis problem in the United States, Am. J Pub Health *41*, 678-686

———, 1953, The knowns and the unknowns in the natural history of encephalitis Proc and Papers 21st Annual Conference of the California Mosquito Control Association

Reeves, W C, and Hammon, W M, 1946, Laboratory transmission of Japanese B encephalitis virus by seven species (three genera) of North American mosquitoes J Exper Med *83*, 185-194

Reeves, W C, Hammon, W M, Doetschman, W H, McClure, H E, and Sather, G, 1955, Studies on mites as vectors of western equine and St. Louis encephalitis viruses in California, Am J Trop Med & Hyg *4*, 90-105

Reeves, W C, Hammon, W M, Lazarus, A S, Brockman, B, McClure, H E, and Doetschman, W H, 1952, The changing picture of encephalitis in the Yakima Valley, Washington, J Infect. Dis *90*, 291-301

Reeves, W C, Sturgeon, J M, French, E M, and Brookman, B, 1954, Transovarian transmission of neutralizing substances to western equine and St Louis encephalitis viruses by avian hosts, J. Infect Dis *95*, 168-178

Richling, E, 1955, Virus meningo encephalitis in Austria 1 Epidemiological features, Bull World Health Organ *12*, 521-534

Robertson, E G, 1952, Murray valley encephalitis Pathological aspects M J Australia *1*, 107-110

Robertson, E. G, and McLorinan, H, 1952, Murray valley encephalitis Clinical aspects, M J Australia *1*, 103-107

de Rodaniche, E, 1956, Isolation of the virus of Ilheus encephalitis from mosquitoes of the genus *Psorophora* captured in Honduras, Am J Trop Med & Hyg *5*, 797-801

de Rodaniche, E, and Galindo, P, 1957, Isolation of Ilheus virus from *Sabethes chloropterus* captured in Guatemala in 1956, Am J Trop Med & Hyg *6*, 686-687

van Rooyen, C E, and Rhodes, A J, 1948, Virus Diseases of Man, New York, Thomas Nelson & Sons

Ross, R W, 1956, The Newala epidemic III The virus Isolation, pathogenic properties and relationship to the epidemic, J. Hyg *54*, 177-191

Rugevich, A S, Ivanova, M G, and Semenova, A N, 1954, Clinical aspects and diagnosis of biundulant virus meningoencephalitis *in* Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 91-101, Medgiz, Leningrad

Sabin, A B., 1947, Epidemic encephalitis in military personnel Isolation of Japanese B virus on Okinawa in 1945, serologic diagnosis, clinical manifestations, epidemiologic aspects and use of mouse-brain vaccine J A M A *133*, 281-293

Sabin, A B, and Buescher, E L, 1950, Unique physico-chemical properties of Japanese B en-

cephalitis virus hemagglutinin, Proc Soc Exper Biol & Med 74, 222-230

Sabin, A B, Duffy, C E, Warren, J, Ward, R, Peck, J L, Jr, and Ruchman, I, 1943, The St Louis and Japanese B types of epidemic encephalitis Development of noninfective vaccines Report of basic data J A M A 122, 477-486

Sabin, A B, Ginder, D R, and Matumoto, M, 1947, Difference in dissemination of the virus of Japanese B encephalitis among domestic animals and human beings in Japan, Am J Hyg 46, 341-355

Scherer, W F, and Syverton, J T, 1954, The viral range in vitro of a malignant human epithelial cell (strain HeLa, Gey) II Studies with encephalitis viruses of the eastern, western, West Nile, St Louis and Japanese B types Am J Path 30, 1075-1084

Shapoval, A N, Dankovskii, N L, Drobyshevskaya, A I, and Sokolov, N N, 1954, Clinical aspects and immunology of attentuated forms of Japanese B encephalitis in Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 305-313, Medgiz, Leningrad

Sharpless, G R, Davies, M C, and Cox, H R, 1950, Antagonistic action of certain neurotropic viruses toward a lymphoid tumor in chickens with resulting immunity, Proc Soc Exper Biol & Med 73, 270-275

Shimizu, T, Kawakami, Y, Fukuhara, S, and Matumoto, M, 1954, Experimental still birth in pregnant swine infected with Japanese encephalitis virus, Jap J Exper Med 24, 363-375

Silber, L A, and Soloviev, V D, 1946, Far Eastern tick-borne spring-summer (spring) encephalitis, Am Review Soviet Med, special supplement, pp 6-80

Smadel, J E, Randall, R, and Warren, J, 1947, Preparation of Japanese encephalitis vaccine Bull U S Army Med Dept 7, 963-972

Smith, C E G, 1956, A virus resembling Russian spring-summer encephalitis virus from an Ixodid tick in Malaya, Nature, London 178, 581-582

———, 1957, A localized outbreak of dengue fever in Kuala Lumpur Serological aspects J Hyg 55, 207-223

Smith, D G, and Herring, A S, 1956, Cultivation and titration of Japanese B encephalitis virus in embryonated eggs, Fed Proc 15, 614

Smith, M G, 1943, Placental transmission of immunity to St Louis encephalitis virus inoculated intraperitoneally in mice, Proc Soc Exper Biol & Med 52, 83 85

Smithburn, K C, 1952, Neutralizing antibodies against certain recently isolated viruses in the sera of human beings residing in East Africa, J Immunol 69, 223-234

———, 1954, Neutralizing antibodies against arthropod-borne viruses in the sera of long-time residents of Malaya and Borneo, Am J Hyg 59, 157-163

Smithburn, K C, and Bugher, J C, 1953, Ultrafiltration of recently isolated neurotropic viruses, J Bact 66, 173-177

Smithburn, K C, and Haddow, A J, 1951, Ntaya virus A hitherto unknown agent isolated from mosquitoes collected in Uganda, Proc Soc Exper Biol & Med 77, 130-133

Smithburn, K C, and Jacobs, H R, 1942, Neutralization-tests against neurotropic viruses with sera collected in Central Africa, J Immunol 44, 9-23

Smithburn, K C, Kerr, J A, and Gatne, P B, 1954a, Neutralizing antibodies against certain viruses in the sera of residents of India, J Immunol 72, 248-257

Smithburn, K C, Kokernot, R H, Weinbren, M P, and de Meillon, B, 1957, Studies on arthropod-borne viruses of Tongaland IX Isolation of Wesselsbron virus from a naturally infected human being and from Aëdes (Banksinella) circumluteolus Theo mosquitoes, South African J M Sc 22, 113-120

Smithburn, K C, Taylor, R M, Rizk, F, and Kader, A, 1954b, Immunity to certain arthropod-borne viruses among indigenous residents of Egypt Am J Trop Med & Hyg 3, 9-18

Smorodintsev, A A, Drobyshevskaya, A I, Il'yenko, V I, Alekseyev, B P, Gulamova, V P, and Fedorchuk, L V, 1954a, Etiology and epidemiology of a new neurotropic virus infection—biundulant meningoencephalitis in Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 4-37, Medgiz, Leningrad

Smorodintsev, A A, Yablonovskaya, L Ya, and Bibikov, D I, 1954b, Recovery of virus and detection of antibodies of Japanese B encephalitis in wild birds in an endemic area in Smorodintsev, A A, Neurotropic Virus Infections (English translation), pp 266-281, Medgiz, Leningrad

South African Institute for Medical Research, Annual Report for 1956, Johannesburg, pp 53-56

Southam, C M, 1956a, Serologic studies of encephalitis in Japan I Hemagglutination-inhibiting, complement-fixing and neutralizing antibody following overt Japanese B encephalitis J Infect Dis 99, 155-162

———, 1956b, Serologic studies of encephalitis in Japan II Inapparent infections by Japanese B encephalitis virus J Infect Dis 99, 163-169

Southam, C M, and Moore, A E, 1951, West Nile, Ilheus and Bunyamwera virus infections in man, Am J Trop Med 31, 724-741

———, 1952, Clinical studies of viruses as antineoplastic agents, with particular reference to Egypt 101 virus, Cancer 5, 1025-1034

———, 1954a, Induced virus infections in man by the Egypt isolates of West Nile virus, Am J Trop Med & Hyg 3, 19-50

———, 1954b, Anti-virus antibody studies following induced infection of man with West Nile, Ilheus and other viruses, J Immunol 72, 446-462

Spence, L P, and Thomas, L, 1958, Application of hemagglutination and complement-fixation techniques to the identification and serological classification of arthropod borne viruses Studies on Chikungunya and Makonde viruses (in preparation)

Stead, F M, and Peters, R F, 1953, The 1952 outbreak of encephalitis in California Vector control aspects California Med *79*, 91-93.

Sulkin, [redacted] E, and Zarafonetis, C, 1947, Influence of anesthesia on experimental neurotropic virus infections II *In vitro* studies with the viruses of western and eastern equine encephalomyelitis, St Louis encephalitis, poliomyelitis (Lansing) and rabies J Exper Med *85*, 559-569

Tahori, A S, Sterk, V V, and Goldblum, N, 1955, Studies on the dynamics of experimental transmission of West Nile virus by *Culex molestus*, Am J Trop Med & Hyg *4*, 1015-1027

Taylor, R M, 1952, Studies on certain viruses isolated in the tropics of Africa and South America Their growth and behavior in the embryonated hen egg J Immunol *68*, 473-494

Taylor, R M, Work, T H, Hurlbut, H S, and Rizk, F, 1956, A study of the ecology of West Nile virus in Egypt, Am J Trop Med & Hyg *5*, 579-620

Tigertt, W D, and Berge, T O, 1957, Japanese B encephalitis, Am J Pub Health *47*, 713-718

Tigertt, W D, and Hammon, W M, 1950, Japanese B encephalitis A complete review of experience on Okinawa 1945-1949, Am J Trop Med *30*, 689-722

van Tongeren, [redacted] A E, 1955-56, Encephalitis in Austria, IV Excretion of virus by milk of the experimentally infected goat, Arch. ges Virusforsch *6*, 158-162

van Tongeren, H A E, Wilterdink, J. D, Wyler, R, and Richling, E, 1955-56, Encephalitis in Austria III A serological survey followed up by an epidemiological study in the endemic region of Styria during the outbreak of 1954 Arch ges Virusforsch *6*, 143-157

Toolan, H W, and Moore, A E, 1952, Oncolytic effect of Egypt virus on a human epidermoid carcinoma grown in X-irradiated rats, Proc Soc Exper Biol & Med *79*, 697-702

Verlinde, J D, van Tongeren, H A E, Pattyn, S R, and Rosenzweig, A, 1955, Virus of meningo-encephalitis in Austria. 3 Pathogenic and immunological properties of the virus Bull World Health Organ *12*, 565-579.

Warren, J., 1946, Epidemic encephalitis in the Far East A review Am J. Trop Med *26*, 417-436.

Warren, J, Weil, M. L, Russ, S B, and Jeffries, H, 1949, Purification of certain viruses by use of protamine sulfate, Proc Soc. Exper. Biol & Med. *72*, 662-664

Weiss, K E, Haig, D A, and Alexander, R A, 1956, Wesselsbron virus—a virus not previously described, associated with abortion in domestic animals, Onderstepoort J Vet. Res *27*, 183-195

Wolf, A., 1950, The pathology of some viral encephalitides *in* Kidd, J [redacted], The Pathogenesis and Pathology of Viral Diseases, pp 194-213, New York, Columbia

Wooley, J G, and Armstrong, C, 1934, The distribution of immunity against encephalitis virus of the St Louis type in the United States as determined by the serum-protection test [redacted] white mice Pub Health Rep *49*, 1495-1505

Work, T H., Hurlbut, H S, and Taylor, R M, 1955, Indigenous wild birds of the Nile delta as potential West Nile virus circulating reservoirs, Am J Trop Med & Hyg *4*, 872-888

Work, T H, and Shah, K V, 1956, Serological diagnosis of Japanese B type of encephalitis in North Arcot district of Madras State, India, with epidemiological notes Indian J M Sc. *10*, 582-592

Work, T H, Trapido, H, Murthy, [redacted] N, Rao, R L, Bhatt, P N, and Kulkarni, K. G., 1957, Kyasanur forest disease III A preliminary report on the nature of the infection and clinical manifestations in human beings Indian J M [redacted] *11*, 619-645

Yaoi, H, Kanazawa, K, Murai, M, and Arakawa, S, 1939, On size of Japanese epidemic encephalitis virus as estimated by "gradocol" membrane, Jap J Exper Med *17*, 375-378

Zimmerman, H M., 1946, The pathology of Japanese [redacted] encephalitis, Am J Path *22*, 965-991

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15

Yellow Fever

(SYNONYMS *Fièvre jaune, fiebre amarilla, febre amarela, Gelbfieber*)

INTRODUCTION

Yellow fever in man is an acute infection caused by a virus The causative agent belongs to Casals' Group B (Chap 12) and is immunologically related to dengue, Uganda S, Zika, Ilheus, etc. In severe cases, yellow fever is characterized by an incubation period of from 3 to 6 days, fever, a pulse rate relatively slow in proportion to the temperature, albuminuria, jaundice, and a tendency to hemorrhage, particularly from the mucosa of the stomach and the gums Clinically, the disease varies from an almost symptomless infection to a severe, rapidly fatal one, death occurring usually within 10 days of onset There are no sequelae, and recovery leaves a lifelong immunity Man acquires the infection by the bite of an infected mosquito The disease occurs in 2 main epidemiologic types In the first, the virus is transmitted from man to man by certain species of mosquito belonging to the genus *Aëdes* of domestic or semidomestic nature The classic urban yellow fever belongs to this type, the virus cycle being man—*Aëdes aegypti*—man In the second, man is only incidentally infected in the course of a virus cycle involving wild animals, particularly monkeys and forest-loving mosquitoes Yellow fever is now endemic in large areas of continental tropical South America and Africa

HISTORY

It is not known whether the original home of yellow fever was in Africa or in America The first epidemic that can be definitely identified as yellow fever occurred in Yucatan in 1648 (Carter, 1931), accounts of previous epidemics are too vague for identification, but there can be little doubt that at this time the disease was very widespread During the 17th, the 18th and the 19th centuries, the disease was widely distributed throughout the Caribbean islands and the adjoining coastal regions of North, Central and South America From this large focus, at times it was transported to more northerly located cities Baltimore, Philadelphia and New York sometimes suffered severe epidemics, which were always confined to the warmer months of the year, disappearing entirely with the onset of cold weather and leaving the cities free until reinfected from outside During this era, yellow fever was essentially a disease of the trade routes of the Atlantic Epidemics were common on sailing vessels where *A aegypti* bred in water vats, and this means of transportation was undoubtedly the method of dissemination On several occasions, yellow fever was taken into the Iberian peninsula where severe epidemics occurred From infected coastal towns, yellow fever was introduced into the heart of North and South America by traffic on the Mississippi and the Amazon.

Some evidence of the severity of yellow fever may be obtained from the figures quoted

Stead, F M, and Peters, R F, 1953, The 1952 outbreak of encephalitis in California Vector control aspects California Med 79, 91-93

Sulkin, S E, and Zarafonetis, C, 1947, Influence of anesthesia on experimental neurotropic virus infections II *In vitro* studies with the viruses of western and eastern equine encephalomyelitis, St Louis encephalitis, poliomyelitis (Lansing) and rabies J Exper Med. 85, 559-569

Tahori, A S, Sterk, V V, and Goldblum, N, 1955, Studies on the dynamics of experimental transmission of West Nile virus by *Culex molestus*, Am J Trop Med & Hyg 4, 1015-1027

Taylor, R M, 1952, Studies on certain viruses isolated in the tropics of Africa and South America Their growth and behavior in the embryonated hen egg J Immunol 68, 473-494

Taylor, R M, Work, T H, Hurlbut, H S, and Rizk, F, 1956, A study of the ecology of West Nile virus in Egypt, Am J Trop Med & Hyg 5, 579-620

Tigertt, W D, and Berge, T O, 1957, Japanese B encephalitis, Am J Pub Health 47, 713-718

Tigertt, W. D, and Hammon, W M, 1950, Japanese B encephalitis A complete review of experience on Okinawa 1945-1949, Am J Trop Med 30, 689-722

van Tongeren, H A E, 1955-56, Encephalitis in Austria, IV Excretion of virus by milk of the experimentally infected goat, Arch ges Virusforsch 6, 158-162

van Tongeren, H A E, Wilterdink, J D, Wyler, R, and Richling, E, 1955-56, Encephalitis in Austria III A serological survey followed up by an epidemiological study in the endemic region of Styria during the outbreak of 1954 Arch ges Virusforsch 6, 143-157

Toolan, H W, and Moore, A E, 1952, Oncolytic effect of Egypt virus on a human epidermoid carcinoma grown in X-irradiated rats, Proc Soc Exper Biol & Med 79, 697-702

Verlinde, J D, van Tongeren, H A E, Pattyn, S R, and Rosenzweig, A, 1955, Virus of meningoencephalitis in Austria, 3, Pathogenic and immunological properties of the virus Bull World Health Organ 12, 565-579.

Warren, J, 1946, Epidemic encephalitis in the Far East A review Am J Trop Med 26, 417-436

Warren, J, Weil, M L, Russ, S. B, and Jeffries, H., 1949, Purification of certain viruses by use of protamine sulfate, Proc Soc Exper Biol & Med. 72, 662-664

Weiss, K E, Haig, D. A, and Alexander, R A., 1956, Wesselsbron virus—a virus not previously described, associated with abortion in domestic animals, Onderstepoort J. Vet Res 27, 183-195

Wolf, A., 1950, The pathology of some viral encephalitides *in* Kidd, J G, The Pathogenesis and Pathology of Viral Diseases, pp 194-213, New York, Columbia.

Wooley, J G, and Armstrong, C., 1934, The distribution of immunity against encephalitis virus of the St Louis type in the United States as determined by the serum-protection test in white mice Pub Health Rep 49, 1495-1505

Work, T H., Hurlbut, H S, and Taylor, R M, 1955, Indigenous wild birds of the Nile delta as potential West Nile virus circulating reservoirs, Am J Trop Med & Hyg 4, 872-888

Work, T H, and Shah, K. V, 1956, Serological diagnosis of Japanese B type of encephalitis in North Arcot district of Madras State, India, with epidemiological notes Indian J M Sc 10, 582-592

Work, T H, Trapido, H, Murthy, D P N, Rao, R L, Bhatt, P N, and Kulkarni, K. G, 1957, Kyasanur forest disease III A preliminary report on the nature of the infection and clinical manifestations in human beings Indian J M. Sc 11, 619-645

Yaoi, H, Kanazawa, K., Murai, M, and Arakawa, S, 1939, On size of Japanese epidemic encephalitis virus as estimated by "gradocol" membrane, Jap J Exper Med 17, 375-378

Zimmerman, H M, 1946, The pathology of Japanese B encephalitis, Am J Path 22, 965-991

of the use of human volunteers (Reed et al, 1911) Of 22 cases produced by the bite of from 1 to 15 infected *Aëdes aegypti* mosquitoes, the mean incubation period was 3 days and 17 hours The extremes were 2 hours less than 3 days, and 2 hours more than 6 days However, the incubation period of yellow fever, acquired from accidental laboratory infection may be 10 days

A patient with a typical infection becomes ill suddenly The clinical course of a severe case is usually divided into the stages of active congestion and stasis with the following signs and symptoms The onset is usually acute and marked by fever, rigor, headache and backache The temperature rises rapidly to reach its maximum on the first or second day. The initial temperature is continuous and seldom exceeds 103 5° F The period of active congestion lasts 3 or 4 days, then the temperature drops The stage of stasis then sets in, and the temperature rises again but seldom reaches its previous maximum The two stages may coalesce without any drop in temperature to mark the transition During the stage of congestion the patient is intensely ill and restless, nausea and vomiting are common. The face is flushed, lips swollen, and eyes injected. The tongue is bright red A tendency to bleeding may be apparent early During the stage of stasis the whole aspect changes The active congestion fades, to be replaced by a venous stasis The face is no longer swollen, and a dusky pallor replaces the bright red of the first stage The gums become swollen and spongy and bleed spontaneously or on light pressure Nausea and vomiting are common The vomited matter usually contains some altered blood, this is the "black vomit" The tendency to hemorrhage is marked, ecchymoses may develop, melena is common The pulse rate is markedly slow in relation to the temperature, this is known as Faget's sign and develops during the first stage, becoming progressively more marked during the second stage The blood pressure is low Albuminuria usually appears toward the end of the period of congestion but becomes very marked during the period of stasis Oliguria or anuria may develop Jaundice appears during the second stage and is seldom very marked, indeed, it may be absent It is more marked as a rule in cases in which the malady is prolonged and in convalescence. Most deaths occur on the sixth or seventh day and seldom later than 10 days after onset. Complications are not frequent Recovery is rapid and complete.

The severity of the disease varies from an extremely mild to a fulminating infection It is possible that many immunizing infections are without symptoms Diagnosis can then be made only by serologic tests However, a study of yellow fever accidentally contracted in the laboratory (Berry and Kitchen, 1931) demonstrated that mild cases show many of the characteristic signs and symptoms of the severe ones In all but a few cases, the saddleback type of temperature curve is apparent The pulse reaction is typical, the peculiar relationship of a slowing heartbeat in a constant or increasing temperature is almost invariable Albuminuria, such a marked feature in severe cases, may be entirely absent in mild ones An important sign in yellow fever infection is the change observed in the white blood cell count There is a steady fall in the number of leukocytes, beginning with the onset of the disease The leukopenia is at its maximum on the fifth or sixth day and is chiefly due to a decrease in the number of neutrophils Lymphopenia is usual and may be marked (Berry and Kitchen, 1931)

The most marked pathologic changes in yellow fever are observed in the liver and the kidneys In the most severe cases there is an almost complete destruction of the parenchymatous cells of the liver, consequently, one finds the metabolic changes associated with extensive liver destruction There is a prothrombin deficiency commensurate with the amount of liver damage This deficiency is probably the cause of the marked hemorrhagic diathesis observed in yellow fever. The bilirubin content of the serum in severe cases is increased According to Elton et al (1955), the kidney lesion is a lower nephron nephrosis resulting from the hemorrhagic diathesis The development of this nephrosis is indicated by the extreme retension of nonprotein nitrogenous metabolites, particularly urea, and may be severe enough to cause death from uremia

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by Reed et al. (1911) It was estimated that there must have been at least 500,000 cases of the disease in the United States during the period between 1793 and 1900. The great epidemic in Spain in 1800 caused 60,000 deaths In Rio de Janeiro, yellow fever was responsible for 23,000 deaths between 1851 and 1883. It was first reported in Cuba in 1640 and was almost continuously present until 1900, causing 35,900 deaths in Havana during the period between 1853 and 1900. During the brief occupation of Cuba by the American forces at the time of the Spanish-American War, 1,575 cases of yellow fever with 231 deaths occurred in the American army, because of this the Yellow Fever Commission was appointed, with Major Walter Reed in charge Modern knowledge of the etiology and the epidemiology of yellow fever stems from the findings of this commission, which clearly demonstrated that the agent responsible for yellow fever passed through bacteria-tight filters, was present in the blood of a patient during the first 3 days of fever, and that the mosquito *Aedes aegypti* was capable of transmitting the disease by bite, provided that a period of 12 days was allowed to lapse after an infective feeding Acting on this information, Gorgas, by applying anti-mosquito measures, was able to eradicate yellow fever from Havana

Following the application of anti-aegypti measures, it was noticed that yellow fever showed a tendency to disappear from neighboring small towns and villages This led to the development by Carter of the "key center" theory, according to which, two factors are necessary to maintain yellow fever in a community (1) a large population of susceptible human beings and (2) a constant supply of the mosquito As considerable evidence was available that an attack of yellow fever produced a lifelong immunity, it was obvious that the requisite number of susceptible persons could occur only where an adequate immigration of people from yellow-fever-free countries took place or in large cities where the newborn supplied this factor On the basis of this theory, the possible key centers were surveyed, and a campaign was set on foot with the object of eradicating yellow fever from the Americas One of the major key centers was Guayaquil Following the eradication of yellow fever from this city the disease disappeared from the entire Pacific coast of South America At the time when it looked as though this campaign was about to be crowned with success a sudden and unexplained epidemic of yellow fever occurred in Rio de Janeiro in 1928.

Stokes et al. (1928), investigating yellow fever in Africa, showed that the rhesus monkey is susceptible to the virus By the use of this experimental animal, it was shown that monkeys indigenous to Africa or South America are susceptible to yellow fever and that mosquitoes, other than *Aëdes aegypti*, under experimental conditions can transmit the virus. These observations first opened up the possibilities of an epidemiology of yellow fever not confined to the cycle, man-*aegypti*-man This possibility was emphasized by the finding of cases of yellow fever in South America in areas where *Aedes aegypti* does not exist and led to the discovery of the epidemiologic entity now known as jungle yellow fever. It has been clearly established that yellow fever virus is maintained in the jungle in South America and Africa in the absence of both man and *A aegypti* and that under such conditions man becomes infected only by close contact with the jungle

The discovery (Theiler, 1930) that white mice are susceptible to an intracerebral inoculation of yellow fever virus, even though the experimental disease in this host does not show the clinical and pathologic pictures of the human malady, led to the development of various protection tests which have proved to be valuable in the study of the epidemiology and the distribution of the disease. The observation that serial passage of the virus in the brains of mice produces a loss of virulence for monkeys led to one method of human vaccination which is still used extensively A more profound modification of the virus was produced by its prolonged maintenance in tissue culture This led to the development of the 17D strain of virus, which is likewise still in use for human vaccination

CLINICAL PICTURE

The most exact information available concerning the incubation period of infection with yellow fever is that obtained as a result

of the use of human volunteers (Reed et al., 1911) Of 22 cases produced by the bite of from 1 to 15 infected *Aedes aegypti* mosquitoes, the mean incubation period was 3 days and 17 hours The extremes were 2 hours less than 3 days, and 2 hours more than 6 days However, the incubation period of yellow fever, acquired from accidental laboratory infection may be 10 days.

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munity produced by previous infection with another virus belonging to Group B. However, among Africans, severe epidemics with a number of deaths, such as that which occurred in the Nuba Mountains in Africa, have been reported (Kirk, 1941) Opinions differ as to whether the disease in children is as severe as in adults It is very difficult to evaluate the over-all mortality of yellow fever. This is usually high in cases in which black vomit and jaundice occur. However, experience has shown that for every case diagnosed, there are usually a great many undiagnosed mild infections as shown by the development of specific neutralizing antibodies Taking into account missed cases, the mortality is probably seldom greater than 5 per cent.

PATHOLOGIC PICTURE

The outstanding macroscopic findings in yellow fever are signs of degeneration in the liver, the kidney and the heart, accompanied by hemorrhages and jaundice The cadaver usually has a livid appearance, due to venous congestion Since the blood in fatal yellow fever remains fluid for a long time, it collects in the dependent portions of the body. Jaundice is always present, seldom well marked The liver is of normal size or slightly enlarged, somewhat yellow in color, and fatty on section, when drained of blood, it has a boxwood color The tense and swollen kidneys are likewise fatty and yellow; the cortex is not clearly demarcated from the medulla Evidence of hemorrhage is usually found, the most frequent site is in the mucosa at the pyloric end of the stomach, where erosions and punctate hemorrhages are present. In very few cases is there a complete absence of altered blood in the stomach contents.

On microscopic examination, the most characteristic lesions are found in the liver (da Rocha Lima, 1912). The liver cells undergo cloudy and fatty degeneration and a distinctive type of necrosis The necrotic cells are mainly confined to the midzone, although in severe cases the necrosis may involve almost the entire lobule. In such cases, however, there will always be found a few normal-appearing cells about the central vein and about the periphery of the lobule. Furthermore, all cells in the midzonal section may not be destroyed, resulting in a spotty distribution of the necrotic areas. The necrosis is hyaline in type and may be confined to part of the cytoplasm, but usually the whole cell is involved. Such necrotic, hyaline cells are known as Councilman bodies. The Kupffer cells are enlarged and may be granular in appearance The sinusoids in the necrotic areas are engorged Actual hemorrhage in the liver is rare In spite of extensive necrosis, the architecture remains intact. During recovery the parenchymatous cells are replaced, leading to a complete restoration of the liver without residual signs of infection such as cirrhosis

The lesions observed in the kidney are not distinctive. As in the liver, there is a complete absence of inflammatory reaction Cloudy and fatty degeneration of the entire kidney tubule, often more marked in the convoluted portion, is usually present The spleen is usually hyperemic, but this is unaccompanied by a leukocytic infiltration (Klotz and Belt, 1930). The most distinctive feature is found in the malpighian corpuscles At first there is a mononucleosis consisting of undifferentiated cells derived from the reticular tissue which persists throughout the entire course of the disease There is a striking loss of lymphocytes False germinal centers are formed. Degeneration is marked throughout the entire organ, which, characterized by cells with vesicular nuclei and waxy-appearing cytoplasm, may lead to actual necrosis, particularly noticeable in the false germinal centers Parallel changes are observed in the lymph nodes Degenerative changes are present throughout the heart

Lesions observed in rhesus monkeys are essentially the same as those in man, namely, degenerative lesions in the liver, the kidney, the spleen and lymph nodes In monkeys that have died of yellow fever following inoculation of unmodified virus, there are no lesions of encephalitis. The encephalitis produced by the neurotropic virus presents no distinctive features, being manifested by necrosis of ganglion cells and perivascular infiltration with round cells. Similar changes are found in the central nervous system of infected mice.

Yellow fever virus infection may produce inclusion bodies, which are intranuclear, acidophilic, variable in size, granular in appearance and irregular in outline. As a rule, they partially surround the nucleolus The

chromatin of the nucleus becomes marginated. These bodies are seen only occasionally in livers of human beings with yellow fever (Cowdry and Kitchen, 1930) The inclusions are found in the nuclei of parenchymatous cells of the liver of infected monkeys and in cells of the brain and the spinal cord of infected mice.

It is apparent that the lesions described above provide an almost complete explanation of the signs and symptoms of yellow fever. Damage of the liver causes hemorrhage and changes in metabolism; albuminuria is due to degenerative changes in the kidney, bradycardia and low blood pressure to involvement of the heart; lymphopenia results from involvement of the lymphoid tissue of the spleen and lymph glands Unfortunately, no studies have been made on the bone marrow of man or monkey dead of yellow fever The marked leukopenia suggests that the myelogenic centers may be affected

Support for most of the above statements has been obtained by a study of the mode of spread and the sites of multiplication of yellow fever virus in rhesus monkeys It has been shown that, following an intradermal inoculation, virus spreads immediately to the local lymph glands where multiplication occurs. After several days, it enters the blood stream and infects the liver, the spleen, the kidneys, bone marrow, and lymph nodes If a monkey survives, virus can still be demonstrated in the lymph nodes, the spleen and bone marrow for several days after the blood has become virus-free There is a clear distinction of the organs involved, depending on the virulence of the virus With highly virulent strains, such as the Asibi, the highest titer of virus is found in the liver On the other hand, the almost avirulent 17D strain can be demonstrated only in spleen, lymph nodes and bone marrow Strains of virus intermediate between these two, for example the mouse-adapted French neurotropic strain, follow more closely the pattern of the Asibi, however, the titers of virus obtained in the liver and blood are not so high (Theiler, 1951)

Yellow fever in monkeys, and presumably in man, is basically an infection of the hemapoietic system and only secondarily involves other organs Almost all lesions can be explained on the basis of infection by and multiplication of the virus Death occurs probably as a direct result of damage done by the virus in the liver and the kidney.

EXPERIMENTAL INFECTION; HOST RANGE

Yellow fever virus shows marked tissue affinities. Unmodified virus is pantropic, showing affinity for all 3 embryonal layers By viscerotropism is meant affinity of the virus for the abdominal viscera, particularly the liver The rhesus monkey is the experimental animal largely used for demonstrating this property All yellow fever viruses exhibit some neurotropism, the common albino mouse is the animal of choice for demonstrating this By various experimental means, both of these attributes can be modified

Strains of yellow fever virus differ in pathogenicity Thus, most strains of African origin are highly pathogenic for rhesus monkeys, almost invariably producing a fatal infection, with death due to an acute necrosis of liver cells In such infections the virus invades the blood stream, where it is present in high concentration. On the other hand, most South American strains when inoculated by extraneural routes have little capacity to destroy visceral cells, rarely producing death from liver necrosis; the only manifestation of infection may be the presence of virus in the blood When a highly viscerotropic strain is inoculated into the brain of a rhesus monkey, death occurs as a result of liver damage and not encephalitis However, the essential neurotropism of such a strain can be shown if, at the time virus is inoculated intracerebrally, the liver is protected by an injection of specific, immune serum A monkey so inoculated will develop fatal encephalitis.

The modification of yellow fever virus is accomplished most readily by serial intracerebral passages in mice (Theiler, 1930) This procedure leads to two predictable results (1) the incubation period and the course of the disease in the mice become shorter, and (2) the pathogenicity for monkeys by parenteral inoculation is diminished. *Pari passu* with this loss of viscerotropism there is an enhancement of neurotropism All yellow fever viruses so far studied, if serially passaged in

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mouse brains, acquire the ability of producing a fatal encephalitis in rhesus monkeys, most readily shown by intracerebral inoculation However, from 5 to 10 per cent of rhesus monkeys inoculated subcutaneously may die of encephalitis The survivors acquire a solid immunity to a subsequent infection with highly viscerotropic virus That the mouse-adapted virus has not entirely lost its viscerotropic affinity can be shown readily by the demonstration of circulating virus In fatal encephalitis, produced either by intracerebral or by parenteral inoculation, the virus is widespread in the peripheral nervous system At the time of death it can be recovered from the brain, the spinal cord, the retina, peripheral nerves, salivary glands and adrenal glands but not from the blood, the cerebiospinal fluid or the liver. Neutralizing antibodies are present at the time of death

A further loss of virulence has been produced by prolonged cultivation in tissue culture (Lloyd et al, 1936) One of the variants, known as the 17D strain, is used extensively for human vaccination (Theiler and Smith, 1937a) and has lost to a considerable extent both its viscerotropic and neurotropic affinities (Theiler and Smith, 1937b) On subcutaneous inoculation of it into rhesus monkeys, a mild systemic infection occurs which can be demonstrated by the presence of minimal amounts of virus in the circulation; on intracerebral inoculation, it produces an encephalitis which is fatal in only about 5 per cent of animals

In addition to the rhesus monkey, it has been found that all South American and African species of monkey so far tested are susceptible to the virus of yellow fever. In most instances, the infection is comparatively mild and accompanied only by a temporary invasion of the blood stream and the development of specific antibodies However, the invasion of the blood is sufficient for mosquitoes to be infected readily by feeding on the monkeys, as a rule, thus indicating the possibility of these animals acting as hosts for the maintenance of virus cycles in nature.

Adult mice are very susceptible to the virus after intracerebral, less after intranasal, and seldom after intraperitoneal inoculation. However, immature mice are highly susceptible to the virus administered by all routes of inoculation. In both adult and infant mice, at the time of death, the virus can be demonstrated only in nervous tissue and in the adrenal gland, an organ which is developmentally a part of the nervous system With age, mice rapidly acquire a resistance to virus inoculated peripherally (Bugher, 1941). This resistance is quite apparent at 9 days of age. Mice younger than this are just as susceptible to neurotropic and viscerotropic strains of virus inoculated subcutaneously as adult mice are to similar strains given intracerebrally. The susceptibility of infant mice to peripheral inoculation has been used to determine the infectivity of mosquitoes in transmission experiments Not all strains of mice are equally susceptible to yellow fever (Sawyer and Lloyd, 1931), of the strains available to these authors, the Swiss strain proved to be the most suitable.

The rabbit and the rat are completely resistant to the virus The guinea pig is, as a rule, susceptible to virus when injected intracerebrally, developing a fatal encephalitis Different strains of guinea pig vary in their response to extraneural inoculation Some are highly susceptible, developing a symptomless, systemic infection, as demonstrated by the presence of virus in the circulation; others appear to be completely resistant (Theiler, 1951)

In the search for possible hosts of jungle yellow fever, the susceptibility of numerous wild animals has been studied (Bugher, 1951) To date, birds, cold-blooded animals, mammals belonging to the orders of *Carnivora* and *Chiroptera*, and most rodents have been shown to be resistant to virus inoculated extraneurally. In most of these groups, very few species have been studied and, as a rule, only adults Animals which have been found to be susceptible include the peccary, several species of edentates (anteaters, armadillos and sloths), a few rodents and all American marsupials Most of these animals are only moderately susceptible, as minimal amounts of circulating virus are present.

The classic urban type of yellow fever is transmitted by *Aëdes aegypti*. The experiments of Reed et al (1911) showed that this mosquito becomes infected by biting a patient during the first 3 days of the disease and that an interval of approximately 12 days

must elapse before it can transmit infection by bite Subsequent work has shown that, under experimental conditions, this extrinsic incubation period can be as short as 4 days and as long as 3 weeks if the mosquitoes are kept at 37° C. or 20° C, respectively (Davis, 1932). A mosquito once infected remains infective for life and harbors the infection without any ill effect There is no evidence that the virus can pass to the next generation through the egg

Evidence is conclusive that yellow fever virus actually multiplies in the mosquito (Whitman, 1937) Following an infective blood-meal the virus in the mosquito diminishes, reaching a minimum during the first week It then increases rapidly until quantities far greater than the amount ingested can be demonstrated Very little is known concerning the relative efficiency of various mosquitoes, shown to be capable of transmitting the virus, to act as intermediate hosts Moreover, it is not known whether the *Aëdes aegypti* mosquito is equally efficient as a vector in its many homes throughout the world Finally, it is not known whether or not all strains of yellow fever virus are equally capable of infecting susceptible mosquitoes From the human experiments of Reed et al (1911), one is warranted in concluding that under the conditions of their work, the *aegypti* mosquitoes used by them became infected readily, as they had no difficulty in transmitting the disease by the bite of from 1 to 15 mosquitoes

The optimum conditions under which mosquitoes become infected is not known. Bates and Roca-García (1945) had difficulty at first in experimentally infecting *Haemagogus* mosquitoes, although numerous infected mosquitoes of this species, capable of producing infection in experimental animals by bites, had been caught in nature Finally, it was discovered that laboratory-fed mosquitoes, if kept at 30° C, are able to transmit the infection readily by bite In these experiments, not only was the extrinsic incubation period shortened by the higher temperature but the percentage of mosquitoes becoming infected was increased Whitman and Antunes (1938) showed that mosquito larvae can be infected by immersion in virus preparations, male and female adults, which are derived from these larvae, are infected, and the females are capable of transmitting the disease by biting

Of African mosquitoes, in addition to *Aëdes aegypti*, the following have been shown experimentally capable of transmitting the virus by bite· *Aëdes luteocephalus*, *A. stokesi*, *A vittatus*, *A africanus*, *A. simpsoni*, *A. taylori*, *A metallicus*, *Eretmopodites chrysogaster*, *Taeniorhynchus africanus* and *Culex thalassius* South American mosquitoes capable of acting as experimental hosts are *Aedes scapularis*, *A. fluviatilis*, *A leucocelaenus*, *Trichoprosopon frontosus*, *Sabethes chloropterus* and various members of the genus *Haemagogus* Species identified as *Haemagogus capricornii*, *H spegazzinii*, *H splendens*, *H uriartei*, *H mesodentatus* and *H equinus* have been shown to be capable of transmitting the virus However, very few of these mosquitoes have actually been incriminated in the epidemiology of yellow fever In South America, yellow fever virus has been isolated on numerous occasions from wild-caught species of *Haemagogus* and several times from *Aedes leucocelaenus* In Africa, *Aëdes simpsoni*, *A. africanus* and *A aegypti* have been found infected in nature (Whitman, 1951)

Mosquitoes are the only bloodsucking arthropods which have been definitely shown to play a part in the epidemiology of yellow fever Miscellaneous experiments have been made with a great variety of other arthropods, such as ticks, mites, fleas, lice, triatomas and bloodsucking flies However, none of these was found capable of transmitting the disease (Whitman, 1951)

ETIOLOGY

Early investigations of the properties of yellow fever virus were made by means of human volunteers, who were used by the American Army Commission under Walter Reed in Havana in 1901 (Reed et al, 1911) Therefore, work was extremely limited, but it was possible to show that the virus passed through filters which retained bacteria When experimental animals became available, it was shown that the etiologic agent of yellow fever is one of the small viruses It readily passes through Seitz as well as all grades of Berkefeld and Chamberland filters By the use of collodion membranes of graded porosity

(gradocol membranes), its diameter has been estimated to be from 17 to 25 mμ The particle size of neurotropic and viscerotropic strains appear to be identical.

The virus of yellow fever is extremely labile; it is readily inactivated by heat and the usual antiseptics It can be preserved in 50 per cent glycerol for several months. The best method for the preservation of the virus is through desiccation while in the frozen state, and storage in a refrigerator, under such conditions it may remain viable for many years. Storage of frozen material in a dry-ice container is convenient and satisfactory, provided that the ampules are sealed Dilution in physiologic salt solution quickly inactivates the virus (Bauer and Mahaffy, 1930). The deleterious action of salt can be prevented by incorporating 10 per cent normal serum or 0 75 per cent bovine plasma albumin in the diluent

Neurotropic yellow fever virus can be cultivated readily in a Maitland-type of tissue culture. However, it was found difficult to adapt an unmodified pantropic strain to tissue culture, the first success was obtained by Lloyd et al (1936), who were able to grow the Asibi strain in a medium containing minced mouse-embryo tissue After some passages, successful cultivation was obtained in a medium containing minced chick embryo In studies to determine whether or not the amount of nervous tissue in the medium had an influence on the neurotropic affinity of the virus, several parallel series of cultures were established containing varying amounts of nervous tissue In the series, containing minimal amounts of nervous tissue, a marked modification of both the viscerotropic and the neurotropic affinities occurred. From these experiments came the 17D strain, now extensively used for vaccination

Yellow fever virus can be maintained in the developing chick embryo. Pantropic, unmodified strains are established with difficulty, but strains which have been maintained in tissue culture or by serial passage in mouse brains are established readily. The chick embryo is used as a routine for the production of vaccine from the 17D strain. Penna and Moussatche (1939) studied the effect of serial passage in the chick embryo on a pantropic strain After a few passages in tissue culture, they established the Asibi strain in chick embryos and noted not only that the viscerotropism of the cultivated strain decreased but that the neurotropism for monkeys was markedly diminished. They were thus able to produce a strain like the 17D. It is noteworthy that in Penna and Moussatche's experiments the marked attentuation occurred in an environment containing large amounts of nervous tissue, for it is known that in the developing chick embryo the brain is one of the main sites of virus multiplication.

Yellow fever virus infection gives rise to neutralizing, complement-fixing and hemagglutination-inhibiting antibodies The presence of neutralizing or protective antibodies is determined by neutralization tests in mice. Various tests are available. In the most sensitive, infant mice and the intraperitoneal route of inoculation are used Adult mice are not susceptible to this route of inoculation However, if at the time of inoculation the brain is damaged by the injection of a sterile solution of starch, infection will take place This is the basis of the intraperitoneal protection test of Sawyer and Lloyd (1931) which was used on a vast scale to map the distribution of yellow fever immunity throughout the world (Smith, 1951) The main drawbacks of the intraperitoneal protection test are the difficulty of standardization and the relatively large quantities of serum required

The intracerebral protection test (Theiler, 1933; Bugher, 1940) consists of mixing the sera to be tested with a suspension of standardized virus stored in sealed ampules at the temperature of dry ice. Several preliminary titrations of the virus preparation make it possible to dilute it so that when mixed with the serum and inoculated in 0 03 cc amounts each mouse receives 100 LD_{50} To determine the end point in virus titrations, the method used is that of Reed and Muench (1938), giving the theoretical dilution that will kill half the number of inoculated mice

The complement-fixation reaction has been studied extensively in yellow fever (Davis, 1931; Lennette and Perlowagora, 1943) Antigens capable of fixing complement are found in the liver and the serum of infected monkeys and in the brains of infected mice The antigen can be demonstrated only in the serum of severely ill monkeys; this probably applies also to human infections

The complement-fixing and protective antibodies are not identical. In man and in experimentally infected monkeys, protective antibodies are sometimes demonstrable before complement-fixing antibodies. Furthermore, in very mild infections, such as that produced by vaccination, complement-fixing antibodies may be absent (Lennette and Perlowagora, 1943). Complement-fixing antibodies disappear comparatively rapidly, consequently, survey for their presence in a population affords information only of yellow fever in the immediate past On the other hand, protective antibodies persist for a long time, if not throughout life, and a survey for them gives an accurate index of the total exposure of a population to yellow fever

DIAGNOSIS

During an epidemic, a diagnosis of yellow fever can often be made on clinical grounds, if the patient shows classic signs and symptoms However, very mild or atypical cases occur in which a diagnosis on clinical grounds usually is impossible Three laboratory procedures are available to establish a diagnosis. (1) isolation of the virus, (2) demonstration of the development of specific antibodies during the course of the disease, and (3) pathologic examination of the liver in fatal cases

For the isolation of virus, serum obtained from a patient, as early in the disease as possible, is inoculated intracerebrally into mice Due to the greater susceptibility of infant mice, these should be employed whenever possible Virus can generally be isolated during the first 4 days of disease On several occasions, successful isolations have been obtained on the fifth and the sixth and on one occasion even on the twelfth day after onset (Downs et al, 1955) In fatal cases virus can at times be isolated from the liver (Anderson and Wattley, 1955) Mice, after a variable incubation period which depends on the concentration of virus in the blood as well as its virulence, develop signs of encephalitis Inasmuch as the signs of disease in the mouse are not specific for yellow fever, the agent thus isolated from a patient must be identified by various means The commonest method is by means of the neutralization test In this, the action of a yellow fever immune serum of known potency is determined on the newly isolated virus If this serum neutralizes the virus under study to approximately the same amount as it neutralizes a known yellow fever strain, it is good evidence that the isolate is a strain of yellow fever.

An alternative method of identification is to prepare a complement-fixing antigen from the brains of infected infant mice and to identify the agent by means of complement-fixation tests If in a box or grid titration a yellow fever immune serum fixes complement to the same extent in the presence of an antigen made from the newly isolated virus as it does in the presence of an equivalent number of units of a known yellow fever antigen, this is very good evidence that the virus is a strain of yellow fever.

In attempts to isolate yellow fever virus from man, it must be borne in mind that some strains of the agent act in a paradoxical manner. Thus, none of a group of mice inoculated with undiluted serum may become infected, whereas the same serum diluted 10- or 100-fold will cause encephalitis in the inoculated animals Therefore, serum to be tested should be inoculated undiluted and in several dilutions into groups of mice By passage in mouse brains, this peculiarity is lost rapidly

Monkeys can also be used for the isolation of yellow fever virus However, it must be realized that the strain of yellow fever may not be lethal for the species of monkey used, consequently, an inoculated animal subsequently is tested for circulating virus at regular intervals by the injection of its serum into mice The virus isolated in the mice is then identified in the manner described above Finally, in the case of survival, the serum of the monkey is tested for specific antibodies Rhesus monkeys have been used most extensively for the isolation of yellow fever virus However, all species of monkey so far studied have been found to be susceptible

As facilities for animal inoculation are not always available, often a diagnosis can be established by the examination of two specimens of serum by serologic means The serologic reaction following yellow fever infection can be of two types, depending upon whether the infection is primary or whether it occurs in an individual who had previously been infected with another agent belonging to Group

B of arbor viruses (Theiler and Casals, 1958) In a primary infection, the antibodies produced are remarkably specific, enabling a diagnosis to be made with assurance, whereas, in the case of a superinfection, antibodies with wide overlaps with other members of the group are produced, making a specific diagnosis virtually impossible. The demonstration of a rise in yellow fever antibodies is of itself not conclusive evidence that the infection was due to yellow fever unless it can be shown that the infection was a primary one For this purpose, the most conclusive evidence is given by complement-fixation tests performed with the convalescent serum In a primary yellow fever infection, the complement-fixing antibodies are often remarkably specific, the convalescent serum fixing complement only with a yellow fever antigen At times there is some fixation with other Group B antigens, but this is always at a much lower titer In the event of a superinfection, the complement-fixation test is completely nonspecific, the convalescent serum fixing complement to a high titer with a wide variety of Group B antigens Hemagglutination-inhibition tests with paired sera supply information of a similar nature In a primary infection HI antibodies of reasonable specificity appear early in the course of the disease The titers of HI antibodies to yellow fever are invariably as high as or higher than antibodies to other Group B agents In superinfections, the pattern of HI antibodies, like the CF antibodies, are not distinctive A further distinction between primary and superinfections is that in the latter antibodies appear very rapidly and develop to a high titer

The diagnosis of fatal yellow fever by the examination of the liver plays a very important part in those countries where the disease is endemic In certain countries, notably Brazil and Colombia, where yellow fever occurs over an enormous area, the systematic study of liver sections of persons who have died of an acute febrile disease of less than 10 days' duration, is a very important function of the Yellow Fever Service (Rickard, 1937). The viscerotome, a simple instrument by which small portions of liver can be obtained from a cadaver, is used This instrument can be employed by a lay person. The portions of liver thus obtained are fixed in formalin solution, embedded in paraffin, and stained with hematoxylin and eosin Diagnosis is based on the presence of typical lesions.

TREATMENT

There is no specific treatment. Complete rest in bed and careful nursing are essential for even the mildest cases Solid food should be withheld Fluids should be forced If persistent vomiting occurs, saline solution may be given by epidermoclysis, and glucose by intravenous injection Cracked ice by mouth may relieve vomiting If this fails, codeine sulfate by hypodermic injection or cocaine hydrochloride by mouth should be given High temperature may be relieved by tepid water sponges To relieve the headache, ice caps may be used, however, it may be necessary to give an analgesic such as codeine sulfate In view of the tendency to constipation, a laxative should be given early in the disease, and thereafter daily enemas should be administered.

EPIDEMIOLOGY

Following the development of the protection test and its application to the study of the world-wide distribution of immunity to yellow fever, several important facts were established. It was shown that yellow fever as it occurred years ago in Cuba, New Orleans, and Panama was immunologically the same as that which is encountered at the present time. The identity of the disease in Africa and America has been clearly established The presence of immunity to yellow fever was shown to be confined to the American and the African continents However, an analysis of the age distribution of protective antibodies indicated that yellow fever is now no longer present in places where it had formerly been prevalent Thus, no evidence was found of any immunity to yellow fever in persons born in Cuba or New Orleans since the last reported epidemics in these places during the first few years of this century.

In Africa and South America the zone of immunity to yellow fever was found to be unexpectedly large. The limits of the area in which antibodies to yellow fever were found in children, and hence areas where the infec-

tion has been present in recent years, comprise the bulk of the tropical zones in the two continents In South America this endemic zone consists of the major portion of the Amazon and the Orinoco basins, in addition to the greater part of Colombia, the Republic of Panama, and the Guianas (Soper, 1938). In this enormous region, *Aedes aegypti* is not universally present so that, clearly, in many places the infection was transmitted in the absence of this vector In Africa, previous to the modern era of research, yellow fever had been reported only along the west coast in a comparatively narrow zone, extending from Dakar to the mouth of the Congo Actually, persons with antibodies capable of neutralizing yellow fever have been found in an area extending from the Atlantic to the Indian Ocean The northern boundary is the Sahara from Dakar to the Eritrean coast on the Red Sea The southern boundary has not yet been clearly demarcated but extends into Northern Rhodesia

Two main epidemiologic entities are distinguished, based on the habits of the vector, the classic urban aegypti-transmitted infection and jungle or sylvan yellow fever, a disease essentially of wild animals and transmitted to man only secondarily

Aedes aegypti is confined to the tropics, with extensions both north and south into the temperate zones This mosquito, like other species belonging to the subgenus *Stegomyia*, is an old-world species and probably was introduced into the western hemisphere during the time of the slave trade The female *Aedes aegypti* prefers to oviposit in water in artificial containers Thus it is essentially a domestic mosquito breeding in houses or in their immediate vicinity This domesticity seems to be more marked in America than in Africa, where it has been captured in small numbers even in primeval forest It is probable that *Aedes aegypti*, like the other species of the subgenus *Stegomyia*, was originally a tree-hole breeder Although this species is present in the tropical zones of the entire world, aegypti-transmitted yellow fever has been reported only in the Americas, Africa and Europe Northward or southward extension of the disease outside the tropics has occurred only during the warm season of the year when climatic conditions were favorable for the mosquito, with the advent of winter epidemics ceased

Being transmitted by *Aëdes aegypti*, a domestic mosquito, urban yellow fever is a household disease affecting all ages and both sexes equally The virus cycle, man-mosquito-man, necessitates an abundance of both the vertebrate and the invertebrate hosts for its maintenance in a community. It follows, therefore, that it is only in relatively large centers of population with a constant influx of susceptibles that this form of yellow fever can persist for any length of time In an aegypti-transmitted epidemic the natural course of events leads to a gradual diminution of the number of susceptibles due to immunization or death Consequently, as the epidemic progresses, more mosquitoes are necessary to maintain the virus cycle Although centers of population with an adequate number of susceptible people are usually large towns, this is not necessarily so An area containing numerous small collections of people with frequent communication between them may remain infected for a long time The infection in such areas wanders from place to place This condition is present in large parts of tropical Africa, and at one time prevailed in Yucatan and in the northeastern portion of Brazil In the last region, owing to the extreme scarcity of water during the dry season, every house had its own water storage. So scarce was water at times that people visiting neighboring villages and farm houses would carry their own drinking water with them In this manner *Aedes aegypti* was widely spread throughout the rural area, and when yellow fever was eradicated from the towns and the villages, it still managed to maintain itself in the comparatively sparsely populated rural area Here, therefore, was the phenomenon of rural yellow fever transmitted by *Aedes aegypti*

The disappearance of yellow fever following the initiation of antimosquito measures was so striking that public health authorities became convinced that the man-*aegypti*-man cycle was the only one. As the war against *Aedes aegypti* progressed, yellow fever apparently disappeared from large areas, and the authorities were justified in their opinions.

However, in spite of success attending the anti-aegypti campaigns, observations were

made that did not fit into the accepted theory. Thus, yellow fever was reported repeatedly from the emerald mines of Muzo in Colombia. Competent investigators came to the conclusion that the disease in Muzo could not be yellow fever, and this conclusion was based largely on the absence of *Aëdes aegypti* in that region Renewed interest in Muzo occurred following aegypti-transmitted epidemics of yellow fever in the nearby towns of Bucaramanga and Socorro. These towns are very isolated, and it was a mystery how the infection was introduced By the use of the protection test, it was shown that immunity to yellow fever was very prevalent in rural, aegypti-free districts, including the Muzo region (Kerr and Patiño Camargo, 1933) Conclusive evidence that yellow fever can occur in rural areas in the absence of *Aedes aegypti* was furnished by the isolation of yellow fever virus during an epidemic which occurred in the Valle do Chanaan, Brazil, in 1932 (Soper et al, 1933) Extensive surveys of immunity to yellow fever clearly demonstrated that the disease was widely distributed in South America in many places where *Aedes aegypti* does not exist

The epidemiologic entity, jungle yellow fever, occurs in South America either in epidemic or endemic form In the endemic form the disease is almost constantly present, and human cases occur year after year The factors concerned in determining human infection in an endemic region have been studied by Soper (1938), Taylor and da Cunha (1946), and Laemmert et al. (1946) Cases of jungle yellow fever are confined almost entirely to adult males This is due to the fact that, as a rule, only the adult male enters the forest to work, clear the jungle, hunt, etc. The infection acquired in the jungle usually is not transmitted to the women and the children, as the common domestic mosquito *Aëdes aegypti* is absent The intimate relationship between the incidence of jungle yellow fever and contact with forest is an adequate explanation of the peculiar sex and age distribution of immunity in a population exposed to the disease, which is quite different from that seen in aegypti-transmitted infections.

Numerous observations in various parts of South and Central America have shown that species of mosquito belonging to the genus *Haemagogus* play an important role in the epidemiology of jungle yellow fever. On many occasions yellow fever virus has been isolated from *Haemagogus* mosquitoes Man is infected only secondarily in a virus cycle in the jungle As yet, the virus cycle or cycles have not been entirely established It is known, however, that various species of monkey play an important role Thus, in most regions where cases of jungle yellow fever have occurred, a considerable proportion of the monkeys show neutralizing antibodies to yellow fever virus Furthermore, in the study of endemic jungle yellow fever in the Ilheus region, yellow fever virus was isolated on 4 separate occasions from marmosets (Laemmert et al, 1946) Extensive investigations in this region failed to produce evidence that any other vertebrate was involved, and *Haemagogus spegazzinii* appeared to be the only vector.

Although it is reasonably certain that primates and *Haemagogus* mosquitoes play an important role in the maintenance of jungle yellow fever virus in nature, there still are many unexplained observations Thus, in the Muzo region, which is a true endemic area, monkeys are either rare or absent. Bugher et al. (1944) have presented evidence that in such areas species of marsupials may act as vertebrate hosts. With certain species of marsupials, yellow fever virus infection can be maintained experimentally by means of mosquitoes. The commonest species, the opossum (*Didelphis marsupialis*), is not very susceptible to the virus. However, Bugher et al (1944) have presented evidence that in jungle yellow fever areas this species shows a high incidence of neutralizing antibodies.

In addition to endemic jungle yellow fever, there is the epidemic type The epidemic form has a tendency to spread farther each year. The most extensive epidemic studied was that of 1933-1938 (Soper, 1938). Presumably originating in the Amazon basin, it spread southeastward through Brazil The spread lasted several years During the colder portions of the year, the infection apparently disappeared, to recommence with the advent of the next summer. The first cases in the new season were often a considerable distance ahead of the last cases of the previous season This wave spread diagonall throughout

Brazil, reaching the Atlantic coast at Rio de Janeiro and then spreading northward along the coast to die out spontaneously It was during this epidemic wave that the genus *Haemagogus* was first shown to play some part in the epidemiology of the disease

An epidemic spread of jungle yellow fever in Central America causing great concern to public health officials is still in progress at the time of writing (Soper, 1955) Previous protection test surveys had indicated that yellow fever, though at one time abundant in Central America, had disappeared from this region. The last epidemic north of the Panama Canal occurred in 1924 in Salvador. The disappearance of yellow fever from this region had been ascribed to the efficacy of *Aedes aegypti* suppression in the key centers The present epidemic wave originated in Eastern Panama in 1948 The disease has moved steadily northward through the rain forest, passing successively through Costa Rica, Nicaragua, Honduras, and Guatemala. The wave of this epidemic was readily followed by a high mortality among the monkeys, particularly the howler monkey This epidemic spread northward, occurring only during the rainy season when *Haemagogus* mosquitoes were abundant Yellow fever virus was isolated from wild-caught *Haemagogus mesodentatus*, *H equinus* and *Sabethes chloropterus* (de Rodaniche and Galindo, 1957) The last mosquito is of special interest because it has a long life and is relatively abundant during the dry season. Thus, it may be able to maintain the virus cycle during the interepidemic seasons (Galindo et al., 1956)

Of unusual interest is the epidemic of yellow fever which occurred in Trinidad in 1954 (Downs, 1955) Apparently, the island had been free of the disease since 1914 The recent epidemic was primarily of the jungle variety, accompanied by a high mortality of howler monkeys. The main vector was *Haemagogus spegazzinii* On 22 occasions, strains of yellow fever were isolated from pools of this species The diagnosis of yellow fever was confirmed in 12 nonfatal cases by the isolation of virus from the blood and in 4 fatal cases by virus isolation from the liver or by histopathologic examination Additional human cases were diagnosed subsequently by serologic methods It was apparent that the epidemic was much more extensive than anyone realized at the time. It is estimated that there were hundreds of cases of unrecognized yellow fever in Trinidad during 1954. *Aedes aegypti* was widely distributed on the island, being present not only in the larger towns but also in the smaller villages situated in the forest. Epidemiologic investigations indicated that several of the rural cases in all probability acquired their infections by the bite of this mosquito However, it is noteworthy that in spite of the prevalence of *Aëdes aegypti* in the towns no epidemic of the classic aegypti-borne type occurred The epidemic on the island developed with great rapidity, and the infection was widely distributed before control measures could be instituted These consisted of mass vaccination and an island-wide anti-aegypti campaign

Somewhat similar conditions are found in Africa. As *Aëdes aegypti* is distributed more widely in Africa than in South America, at times it is difficult to make a clear distinction between the two epidemiologic types of the disease However, conclusive evidence has been obtained that primates act as vertebrate hosts of the virus The most likely invertebrate host in the forest is *Aedes africanus*, which is most active at twilight and at night and prefers the upper foliage As in the Americas, man plays no part in the maintenance of the virus cycle in the jungle Because the habitat of *Aedes africanus* is in the forest canopy, it is extremely unlikely that this mosquito infects man in the forest

The sequence of events seems to be as follows Certain species of monkey have the habit of raiding human plantations On the edge of the forest and on the plantation, another mosquito, *Aedes simpsoni*, occurs, which is a plant-axil breeder Presumably, this mosquito becomes infected from monkeys and then transmits the disease to man. Under experimental conditions both *Aedes africanus* and *Aedes simpsoni* are efficient vectors of the disease Yellow fever virus has been isolated from *A simpsoni* caught in a region where cases of yellow fever were occurring (Smithburn and Haddow, 1949) and from *Aedes africanus* caught in the uninhabited forest

in sufficient numbers to be considered as a possible vector.

The classic urban, aegypti-borne yellow fever is comparatively common in Africa. This is the type which has been frequently observed in West Africa. Extensive epidemics of rural yellow fever also occur. This type may or may not be transmitted by *Aedes aegypti*. The most extensive epidemic of this type, and one of the most severe described in recent years, is that which occurred in the Nuba Mountains in the Anglo-Egyptian Sudan in 1940 (Kirk, 1941). Over 15,000 cases were recorded, with more than 1,500 deaths. A survey for immunity to yellow fever after the epidemic indicated that approximately 40,000 cases must have occurred. *Aedes aegypti*, although present, was, on epidemiologic grounds, not the principal vector. *Aëdes vittatus*, *Aëdes taylori*, and *Aedes metallicus*, all known to be efficient vectors under experimental conditions, were present in the region, and one of them was probably the main vector in the epidemic.

Recent investigations, using the infant mouse neutralization test, have shown conclusively that sera from individuals who have previously experienced infections with a variety of Group B agents such as dengue, West Nile, Ilheus and Japanese B have the capacity of neutralizing yellow fever virus. These observations strongly suggest that individuals who have experienced a Group B virus infection are relatively immune to yellow fever and may account for many of the mild clinical cases. These observations also suggest the possibility that, in a population largely immune to dengue or other Group B viruses, it may be difficult or even impossible for an epidemic of aegypti-transmitted yellow fever to occur. During the recent epidemic of jungle yellow fever in Trinidad (Downs, 1955), it was noteworthy that in spite of the fact that *Aëdes aegypti* was abundant in Port-of-Spain, the capital, no aegypti-borne epidemic occurred. The incidence of antibodies to dengue is very high in Port-of-Spain. The marked immunologic overlaps among the Group B viruses may afford an explanation for the absence of aegypti-borne yellow fever in regions of the world where this mosquito is abundant and, consequently, where conditions appear to be favorable for the maintenance of yellow fever infections.

CONTROL MEASURES

Prior to the development of methods of vaccination anti-aegypti measures were the only ones available for the control of yellow fever. Their efficacy was clearly established by Gorgas and others immediately following the demonstration that *Aedes aegypti* acted as a vector. Following the successes in Havana, New Orleans, Panama, Río de Janeiro and Guayaquil, these methods became firmly established. The measures employed were at first confined to the control of breeding. This was achieved by a weekly search for larvae in and in the immediate vicinity of every house. Inspectors had authority to pour oil into any container found to harbor *Aedes aegypti* larvae. This procedure has a 2-fold effect: (1) the oil kills the larvae, and (2) the householder is impelled to clean the container. The difficulty of cleaning receptacles after oil has been poured into them induces the householder to do everything possible to prevent mosquito-breeding. Such weekly inspection and eradication of all breeding places are adequate to reduce the number of adult mosquitoes below a level where transmission is possible. However, this procedure is not sufficient to eradicate the mosquito entirely, because hidden breeding foci are not discovered during routine inspections. In order to find these, specially trained inspectors are employed. Their function is to make captures of adult mosquitoes in every

in that house in which the largest number of adult mosquitoes is found. By these means it is possible to find every breeding focus in a town and, consequently, to eradicate *Aëdes aegypti*. From an administrative point of view, it has been found cheaper to eradicate *Aedes aegypti* than to keep the mosquito population below a critical level. The latter necessitates a permanent inspection force to prevent the re-establishment of a large mosquito population. Following eradication, reinfestation does not occur readily, and a skeleton force is suf-

ficient to verify the continued absence of the mosquito, particularly if similar anti-aegypti measures are employed in nearby towns In Brazil, anti-aegypti measures have been employed so extensively that for several years no cases of yellow fever have been reported as being aegypti-borne. In fact, the species has been eradicated from entire states A very active campaign is being waged at present in an effort to eradicate *Aedes aegypti* from the Americas

In Africa the situation in regard to the control of *Aëdes aegypti* is not so favorable In the first place, *Aëdes aegypti* is distributed far more widely Secondly, this species does not seem to be as domestic as in the Americas In fact, specimens have been captured in primeval forest Furthermore, in tropical Africa, there are very few large centers of population, as a rule, natives live in small villages In large towns, the installation of a pipe water supply is one of the most efficacious methods of *aegypti* control

The development of the newer insecticides has proved of great value to the sanitarian interested in yellow fever control Thus, DDT would appear to be almost ideal for the control of *Aedes aegypti*, applied as a residual spray to the walls of all houses or to the outside and the inside of household water containers at infrequent intervals, it will reduce the number of mosquitoes so significantly that this procedure alone is a very effective and inexpensive method of control The extensive use of a DDT spray is probably the method of choice in combating a severe aegypti-borne epidemic of yellow fever Marked reduction of adult mosquitoes should occur immediately, whereas experience has shown that antilarval measures require approximately 6 weeks to reduce the mosquito population below the critical level (Antunes, 1948)

Two strains of attenuated yellow fever virus are currently in use for human vaccination These are known as the French neurotropic and the 17D strains The French neurotropic virus is used extensively by the French in their West and Central African colonies (Peltier et al, 1940) The original French strain of yellow fever virus was isolated from a Syrian in Dakar in 1929 This is the strain which was first shown to be pathogenic for mice by intracerebral inoculation (Theiler, 1930). By serial passage, this virus became more pathogenic for these rodents but lost entirely its capacity for producing fatal visceral yellow fever in rhesus monkeys The method of vaccination as employed by the French consists in the use of this mouse-adapted virus

Other investigators, largely as a result of animal experiments, considered this strain too virulent for human use Consequently, a method of vaccination was evolved in which the subcutaneous inoculation of the French neurotropic virus was preceded by an injection of human yellow fever immune serum (Sawyer, et al, 1932). This method proved to be effective but cumbersome and impracticable for large-scale immunization Consequently, efforts were made to produce a strain more modified than the French neurotropic. This was achieved by prolonged cultivation of yellow fever virus in tissue culture This produced the 17D strain

For the production of vaccine, developing chick embryos are inoculated (Penna, 1956) After 4 days' incubation at 37° C. the embryos are harvested and reduced to a pulp, either in a ball mill or a Waring Blendor The infected chick-embryo juice, after the addition of some sterile distilled water, is then measured into ampules and desiccated while in the frozen state Before sealing, the ampules are filled with dry nitrogen gas Vaccine is stored in a refrigerator For use, the vaccine is reconstituted in the requisite amount of sterile salt solution, and 0 5 cc is injected subcutaneously As the virus in the vaccine is active, only one inoculation is necessary Experiments have shown that very minute quantities of active virus are capable of producing immunity in man (Fox et al, 1943), the minimum inoculum for successful vaccination has been set at 500 M L D (for mice) The reactions following vaccination usually are remarkably mild In approximately 5 per cent of persons there is a reaction on about the seventh day (Smith et al, 1938), this consists of malaise, headache, backache and a slight elevation of temperature, and, usually lasting about a day, it is seldom severe enough to interfere with a person's daily routine On rare occasions encephalitic symptoms have occurred following vaccination with 17D These cases occurred in infants who all recovered

without any sequelae (Stuart, 1956). Immunity, as determined by the demonstration of specific neutralizing antibodies, is manifest by the seventh to the ninth day after vaccination The titer of antibodies in persons vaccinated with 17D virus is, as a rule, low In fact, as evaluated by the standard protection test, there are always approximately 5 or 10 per cent of people whose sera, according to the criteria of the test, must be considered negative However, by the use of more delicate tests it can be shown that these are not truly negative but contain specific neutralizing antibodies, though in minimal amounts The duration of immunity following vaccination has not been determined. However, it is known that the number of persons showing antibodies is not significantly decreased after 6 years (Dick and Smithburn, 1949) There is some evidence that the immune response in children is less than in adults.

The method of vaccination used at present by the French consists of applying the vaccine suspended in a solution of gum arabic to the scarified skin (Peltier et al , 1940, Durieux, 1956). The vaccine is issued in the form of dried mouse brain infected with the French neurotropic strain of virus Suspension of the vaccine in gum arabic is made immediately before vaccination Often dried vaccinia virus is mixed with the gum at the same time, and the individuals are thus vaccinated against 2 diseases simultaneously, viz , yellow fever and smallpox. The development of specific antibodies to yellow fever following the French method of vaccination is better than that following the use of 17D However, the number of reactions is greater, being approximately 15 per cent Serious reactions are rare, but several very severe episodes have occurred following the use of the French neurotropic vaccine The most thoroughly studied of these episodes occurred in Nigeria Following the immunization of 42,000 Africans, 83 cases of encephalitis occurred within a 24-day period From the brain tissues of 4 cases that died of encephalitis 14. 14, 18 and 19 days after vaccination, strains of yellow fever virus were isolated which were indistinguishable in pathogenicity from the vaccine virus These studies indicate that on occasion the French neurotropic vaccine may produce a fatal encephalitis (Macnamara, 1955) However, it is noteworthy that the number of severe reactions are far less than the results of experiments on monkeys would have led one to expect. This method of vaccination has been used extensively in the African colonies The ease of administration and the cheapness of manufacture are of great importance.

The most conclusive evidence that vaccination is an effective prophylactic measure is supplied by the fact that, since the introduction of vaccination, no accidental cases of yellow fever have occurred in laboratory workers. Prior to the development of a vaccine, these infections were extremely common and in several cases even fatal Of similar nature is the observation that workers, investigating jungle yellow fever and using themselves as mosquito bait, on numerous occasions have isolated virus from *Haemagogus* mosquitoes caught in this manner. All such workers had been vaccinated and did not contract yellow fever. Observations such as this are good evidence that vaccination is highly efficacious.

To date, the results of mass vaccination have been satisfactory. In areas in Colombia where jungle yellow fever is prevalent and where efforts were made to vaccinate the entire population, fatal cases of yellow fever continued to occur But it is noteworthy that all these cases were in the small portion of the population which had not been vaccinated Vaccination is the only method available for the protection of persons exposed to the risk of jungle yellow fever In Africa, where anti-aegypti measures are to a large extent impracticable, mass vaccination is of particular value. In fact, this is the policy of the French government, which has embarked on a plan of vaccinating every person in their colonies every 4 years wherever yellow fever is present. The ease of manufacture and administration of their vaccine makes such a plan feasible.

REFERENCES

Anderson, C R, and Wattley, G, 1955, The isolation of yellow fever virus from human liver obtained at autopsy, Tr. Roy. Soc Trop Med & Hyg 49, 580-581

Antunes, W S, 1948, Field control in yellow fever,

Proc Internat Cong Trop Med & Malaria *1*, 498-505

Bates, M, and Roca-García, M, 1945, Laboratory studies of the Saimiri-Haemogogus cycle of jungle yellow fever, Am J Trop Med *25*, 203-216

Bauer, J H, and Mahaffy, A F, 1930, Studies on the filtrability of yellow fever virus, Am J Hyg *12*, 175-195

Berry, G P, and Kitchen, S F, 1931, Yellow fever accidentally contracted in the laboratory a study of seven cases, Am J Trop Med *11*, 365-434

Bugher, J C, 1940, The demonstration of yellow fever antibodies in animal sera by the intracerebral protection test in mice, Am J Trop Med *20*, 809 841

———, 1941, The use of baby mice in yellow fever studies, Am J Trop Med *21*, 299-307

———, 1951, The mammalian host in yellow fever *in* Yellow Fever, edited by George K Strode, New York, McGraw-Hill

Bugher, J C, Boshell-Manrique, J, Roca-García, M, and Osorno-Mesa, E, 1944, Epidemiology of jungle yellow fever in eastern Colombia, Am J Hyg *39*, 16-51

Carter, H R, 1931, Yellow Fever, An Epidemiological and Historical Study of its Place of Origin, Baltimore, Williams & Wilkins

Cowdry, E V, and Kitchen, S F, 1930, Intranuclear inclusions in yellow fever, Am J Hyg *11*, 227-299

Davis, N E, 1931, Complement fixation in yellow fever in monkey and in man, Am J Hyg *13*, 79-128

Davis, N C, 1932, The effect of various temperatures in modifying the extrinsic period of the yellow fever virus in *Aëdes aegypti*, Am J Hyg *16*, 163-176

Dick, G W A, and Smithburn, K C, 1949, Immunity to yellow fever six years after vaccination, Am J Trop Med *29*, 57-61

Downs, W G, 1955, Epidemiological notes in connection with the 1954 epidemic of yellow fever in Trinidad, B W I, *in* Yellow Fever A symposium in commemoration of Carlos Juan Finlay The Jefferson Medical College of Philadelphia, pp 22-23, September, 1955

Downs, W G, Anderson, C R, Spence, L, 1955, The isolation of yellow fever virus from a human patient on the twelfth day of disease, Tr Roy Soc Trop Med & Hyg *49*, 577-579

Durieux, C, 1956, Preparation of yellow fever vaccine in the Institut Pasteur, Dakar *in* Yellow Fever Vaccination, Geneva, World Health Organ

Elton, N W, Romero A and Trejos, A, 1955, Clinical pathology of yellow fever, Am J Clin Path *25*, 135-146

Fox, J P, Kossobudzki, S L, and da Cunha, J F, 1943, Field studies on the immune response to 17D yellow fever virus Relation to virus substrain, dose and route of inoculation Am J Hyg *38*, 113-138

Galindo, P, Rodaniche, E de, and Trapido, H, 1956, Experimental transmission of yellow fever virus by Central American species of *Haemagogus* and *Sabethes chloropterus*, Am J Trop Med & Hyg *5*, 1022-1031

Kerr, J A, and Patiño Camargo, L, 1933, Investigaciones sobre fiebre amarilla en Muzo y Santander Revista de Higiene, Bogota *2*, 63-83

Kirk, R, 1941, An epidemic of yellow fever in the Nuba Mountains, Anglo-Egyptian Sudan Ann Trop Med *35*, 67-112

Klotz, O, and Belt, T H, 1930, The pathology of the spleen in yellow fever, Am J Path *6*, 655-662

Laemmert, H W, de Castro Ferreira, L, and Taylor, R M, 1946, An epidemiological study of jungle yellow fever in an endemic area in Brazil Part II Investigations of vertebrate hosts and arthropod vectors Am J Trop Med *26* (Suppl) 23-69

Lennette, E H, and Perlowagora, A, 1943, The complement fixation test in the diagnosis of yellow fever Use of infectious mouse brain as antigen Am J Trop Med *23*, 481-504

Lloyd, W, Theiler, M, and Ricci, N I, 1936, Modification of the virulence of yellow fever virus by cultivation in tissues *in vitro*, Tr Roy Soc Trop Med & Hyg *29*, 481-529

Macnamara, F N, 1953, Reactions following neurotropic yellow fever vaccine given by scarification in Nigeria, Tr Roy Soc Trop Med & Hyg *47*, 199-208

Peltier, M, Durieux, C, Jonchere, H, and Arquie, E, 1940, Vaccination mixte contre la fievre jaune et la variole sur des populations indigene du Sénegal Ann Inst Pasteur *65*, 146-169

Penna, H A, 1956, Production of 17D yellow fever vaccine *in* Yellow Fever Vaccination, Geneva, World Health Organ

Penna, H A, and Moussatche, H, 1939, Modificacão do virus de febre amarella por passagens em serie no embryao de gallinha em desenvolvimento, Brasil-med *53*, 903 905

Reed, W, Carroll, J, Agramonte, A, and Lazear, J W, 1911, Yellow Fever A compilation of various publications, U S 61st Cong, 3d sess Senate, Doc no 822, Washington

Reed, L J, and Muench, H, 1938, A simple method of estimating fifty per cent endpoints, Am J Hyg *27*, 493-497

Rickard, E R, 1937, The organization of the viscerotome service of the Brazilian cooperative yellow fever service, Am J Trop Med *17*, 163-190

da Rocha Lima, H, 1912, Zur pathologischen Anatomie des Gelbfiebers, Verhandl deutsch path Gesellsch *15*, 163-181

Rodaniche, E de, and Galindo, P, 1957, Isolation of yellow fever virus from Hae...

945-969

Sawyer, W A, and Lloyd, W, 1931, The use of mice in tests of immunity against yellow fever, J Exper Med *54*, 533-555

without any sequelae (Stuart, 1956) Immunity, as determined by the demonstration of specific neutralizing antibodies, is manifest by the seventh to the ninth day after vaccination The titer of antibodies in persons vaccinated with 17D virus is, as a rule, low. In fact, as evaluated by the standard protection test, there are always approximately 5 or 10 per cent of people whose sera, according to the criteria of the test, must be considered negative However, by the use of more delicate tests it can be shown that these are not truly negative but contain specific neutralizing antibodies, though in minimal amounts The duration of immunity following vaccination has not been determined However, it is known that the number of persons showing antibodies is not significantly decreased after 6 years (Dick and Smithburn, 1949) There is some evidence that the immune response in children is less than in adults

The method of vaccination used at present by the French consists of applying the vaccine suspended in a solution of gum arabic to the scarified skin (Peltier et al., 1940, Durieux, 1956). The vaccine is issued in the form of dried mouse brain infected with the French neurotropic strain of virus Suspension of the vaccine in gum arabic is made immediately before vaccination Often dried vaccinia virus is mixed with the gum at the same time, and the individuals are thus vaccinated against 2 diseases simultaneously, viz, yellow fever and smallpox The development of specific antibodies to yellow fever following the French method of vaccination is better than that following the use of 17D However, the number of reactions is greater, being approximately 15 per cent Serious reactions are rare, but several very severe episodes have occurred following the use of the French neurotropic vaccine. The most thoroughly studied of these episodes occurred in Nigeria Following the immunization of 42,000 Africans, 83 cases of encephalitis occurred within a 24-day period From the brain tissues of 4 cases that died of encephalitis 14, 14, 18 and 19 days after vaccination, strains of yellow fever virus were isolated which were indistinguishable in pathogenicity from the vaccine virus. These studies indicate that on occasion the French neurotropic vaccine may produce a fatal encephalitis (Macnamara, 1953). However, it is noteworthy that the number of severe reactions are far less than the results of experiments on monkeys would have led one to expect. This method of vaccination has been used extensively in the African colonies The ease of administration and the cheapness of manufacture are of great importance.

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REFERENCES

Anderson, C R, and Wattley, G, 1955, The isolation of yellow fever virus from human liver obtained at autopsy, Tr Roy Soc Trop Med & Hyg 49, 580-581

Antunes, W S, 1948, Field control in yellow fever,

Proc Internat Cong Trop Med & Malaria *1*, 498-505

Bates, M, and Roca-Garcia, M, 1945, Laboratory studies of the Saimiri-Haemogogus cycle of jungle yellow fever, Am J Trop Med *25*, 203-216

Bauer, J H, and Mahaffy, A F, 1930, Studies on the filtrability of yellow fever virus, Am J Hyg *12*, 175-195

Berry, G P, and Kitchen, S F, 1931, Yellow fever accidentally contracted in the laboratory a study of seven cases, Am J Trop Med *11*, 365-434

Bugher, J C, 1940, The demonstration of yellow fever antibodies in animal sera by the intracerebral protection test in mice, Am J Trop Med *20*, 809-841

———, 1941, The use of baby mice in yellow fever studies, Am J Trop Med *21*, 299-307

———, 1951, The mammalian host in yellow fever *in* Yellow Fever, edited by George K Strode, New York, McGraw-Hill

Bugher, J C, Boshell-Manrique, J, Roca-Garcia, M, and Osorno-Mesa, E, 1944, Epidemiology of jungle yellow fever in eastern Colombia, Am J Hyg *39*, 16-51

Carter, H R, 1931, Yellow Fever, An Epidemiological and Historical Study of its Place of Origin, Baltimore, Williams & Wilkins

Cowdry, E V, and Kitchen, S F, 1930, Intranuclear inclusions in yellow fever, Am J Hyg *11*, 227-299

Davis, N E, 1931, Complement fixation in yellow fever in monkey and in man, Am J Hyg *13*, 79-128

Davis, N C, 1932, The effect of various temperatures in modifying the extrinsic period of the yellow fever virus in *Aëdes aegypti*, Am J Hyg *16*, 163-176

Dick, G W A, and Smithburn, K C, 1949, Immunity to yellow fever six years after vaccination, Am J Trop Med *29*, 57-61

Downs, W G, 1955, Epidemiological notes in connection with the 1954 epidemic of yellow fever in Trinidad, B W I, *in* Yellow Fever A symposium in commemoration of Carlos Juan Finlay, The Jefferson Medical College of Philadelphia, pp 22-23, September, 1955

Downs, W G, Anderson, C R, Spence, L, 1955, The isolation of yellow fever virus from a human patient on the twelfth day of disease, Tr Roy Soc Trop Med & Hyg 49, 577-579

Durieux, C, 1956, Preparation of yellow fever vaccine in the Institut Pasteur, Dakar *in* Yellow Fever Vaccination, Geneva, World Health Organ

Elton, N W, Romero A and Trejos, A, 1955, Clinical pathology of yellow fever, Am J Clin Path *25*, 135-146

Fox, J P, Kossobudzki, S L, and da Cunha, J F, 1943, Field studies on the immune response to 17D yellow fever virus Relation to virus substrain, dose and route of inoculation Am J Hyg *38*, 113-138

Galindo, P, Rodaniche, E de, and Trapido, H, 1956, Experimental transmission of yellow fever virus by Central American species of *Haemagogus* and *Sabethes chloropterus*, Am J Trop Med & Hyg *5*, 1022-1031

Kerr, J A, and Patiño Camargo, L, 1933, Investigaciones sobre fiebre amarilla en Muzo y Santander Revista de Higiene, Bogota *2*, 63-83

Kirk, R, 1941, An epidemic of yellow fever in the Nuba Mountains, Anglo-Egyptian Sudan Ann Trop Med *35*, 67-112

Klotz, O, and Belt, T H, 1930, The pathology of the spleen in yellow fever, Am J Path *6*, 655-662

Laemmert, H W, de Castro Ferreira, L, and Taylor, R M, 1946, An epidemiological study of jungle yellow fever in an endemic area in Brazil Part II Investigations of vertebrate hosts and arthropod vectors Am J Trop Med *26* (Suppl) 23-69

Lennette, E H, and Perlowagora, A, 1943, The complement fixation test in the diagnosis of yellow fever Use of infectious mouse brain as antigen Am J Trop Med *23*, 481-504

Lloyd, W, Theiler, M, and Ricci, N I, 1936, Modification of the virulence of yellow fever virus by

199-203

Peltier, M, Durieux, C, Jonchère, H, and Arquie, E, 1940, Vaccination mixte contre la fièvre jaune et la variole sur des populations indigene du [illegible]négal Ann Inst Pasteur *65*, 146-169

Penna, H A, 1956, Production of 17D yellow fever vaccine *in* Yellow Fever Vaccination, Geneva, World Health Organ

Penna, H A, and Moussatché, H, 1939, Modificação do virus de febre amarella por passagens em serie no embryão de gallinha em desenvolvimento, Brasil-méd *53*, 903-905

Reed, W, Carroll, J, Agramonte, A, and Lazear, J W, 1911, Yellow Fever A compilation of various publications, U S 61st Cong, 3d sess Senate, Doc no 822, Washington

Reed, L J, and Muench, H, 1938, A simple method of estimating fifty per cent endpoints, Am J Hyg *27*, 493-497

Rickard, E R, 1937, The organization of the viscerotome service of the Brazilian cooperative yellow fever service, Am J Trop Med *17*, 163-190

da Rocha Lima, H, 1912, Zur pathologischen Anatomie des Gelbfiebers, Verhandl deutsch path Gesellsch *15*, 163-181

Rodaniche, E de, and Galindo, P, 1957, Isolation of yellow fever virus from *Haemagogus mesodentatus, H equinus* and *Sabethes chloropterus* captured in Guatemala in 1956, Am J Trop Med & Hyg *6*, 232-237

Sawyer, W A, Kitchen, S F, and Lloyd, W, 1932, Vaccination against yellow fever with immune serum and virus fixed for mice, J Exper Med *55*, 945-969

Sawyer, W A, and Lloyd, W, 1931, The use of mice in tests of immunity against yellow fever, J Exper Med *54*, 533-555

Smith, H H, 1951, Controlling yellow fever in Yellow Fever, edited by George K Strode, New York, McGraw-Hill

Smith, H H, Penna, H A, and Paoliello, A, 1938, Yellow fever vaccination with cultured virus (17D) without immune serum, Am J Trop Med *18*, 437-468

Smithburn, K C, Haddow, A J, and Lumsden, W H R, 1949, An outbreak of sylvan yellow fever in Uganda with *Aëdes (Stegomyia) africanus* Theobald as principal vector and insect host of virus, Ann Trop Med *43*, 74-89

Soper, F L, 1938, Yellow Fever The present situation (October, 1938) with special reference to South America, Tr Roy Soc Trop Med & Hyg *32*, 297-332

———, 1955, Review of the Yellow Fever menace, Am J Trop Med & Hyg *4*, 573-582

Soper, F L, Penna, H, Cardoso, E, Serafim, J, Jr, Frobisher, M, Jr, and Pinheiro, J. 1933, Yellow fever without *Aedes aegypti* Study of a rural epidemic in the Valle do Chanaan, Espirito Santo, Brazil, 1932, Am J Hyg *18*, 555-587.

Stokes, A, Bauer, J H, and Hudson, N P, 1928, Experimental transmission of yellow fever to laboratory animals, Am J Trop Med *8*, 103-164

Stuart, G, 1956, Reactions following vaccination against yellow fever in Yellow Fever Vaccination, Geneva, World Health Organ

Taylor, R M, and Fonseca da Cunha, J, 1946, An epidemiological study of jungle yellow fever in an endemic area in Brazil Part I Epidemiology of human infections Am J. Trop Med *26* (Suppl) 1-21

Theiler, M, 1930, Studies on the action of yellow fever virus in mice, Ann Trop Med *24*, 249-272

———, 1933, A yellow fever protection test in mice by intracerebral injection, Ann Trop Med *27*, 57-77.

———, 1951, The virus in Yellow Fever, edited by George K Strode, New York, McGraw-Hill

Theiler, M, and Casals, J, 1958, The serological reactions in yellow fever Am J Trop Med & Hyg In press

Theiler, M, and Smith, H H, 1937a, The use of yellow fever virus modified by *in vitro* cultivation for human immunization, J Exper Med *65*, 787-800

———, 1937b, The effect of prolonged cultivation *in vitro* upon the pathogenicity of yellow fever virus, J Exper Med *65*, 767-786

Whitman, L, 1937, The multiplication of the virus of yellow fever in *Aedes aegypti*, J Exper Med *66*, 133-143

———, 1951, The arthropod vectors in Yellow Fever, edited by George K Strode, New York, McGraw-Hill

Whitman, L, and Antunes, P C A, 1938, Studies on *Aedes aegypti* infected in the larval stage with the virus of yellow fever, Proc Soc Exper Biol & Med *37*, 664-666

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16

Dengue

(SYNONYMS Break-bone fever, dandy fever, *denguero*, bouquet fever, giraffe fever, polka fever, 5-day fever, 7-day fever)

INTRODUCTION

Dengue is an infectious, mosquito-transmitted disease of virus etiology, characterized by fever, pain in various parts of the body, prostration, rash, lymphadenopathy and leukopenia

HISTORY

David Bylon is credited with the first description of an epidemic of this disease, which he called "joint fever" in Batavia, Java, in 1779 In 1780 Benjamin Rush described an epidemic in Philadelphia, Pa , under the name of "bilious remitting fever " During the 19th century, innumerable reports appeared of epidemics in subtropical and tropical regions throughout the world, and the name "dengue" was accepted for standard usage by the Royal College of Physicians of London in 1869 Some of the largest epidemics in history have occurred in the United States, Australia, Greece and Japan since 1920 During the 1922 epidemic in the U S it was estimated that between 1 and 2 million people had dengue in the southern states The Queensland-New South Wales epidemic of 1925-1926 attacked approximately 560,000 persons, and another large epidemic occurred in Australia in 1942 (Lumley and Taylor, 1943) During the 1927-1928 epidemic in Greece, the total number of cases probably exceeded a million From 1942 to 1945, the main ports of Japan had large, yearly epidemics of dengue with an estimated number of cases of from 1 to 2 million, the city of Osaka alone having one third to one half of its population attacked in 1944 (Sabin, 1952) Although only 84 090 cases of dengue were officially reported during the war years in U S. Army personnel, the total number of cases was undoubtedly much larger

authors also proved that *Aedes albopictus* is a true vector Ashburn and Craig, 1907, provided the first evidence that the etiologic agent of dengue is filterable and ultramicroscopic. The demonstration that dengue virus can produce inapparent infection in certain species of monkeys was followed by proof that certain monkeys may be infected *in nature*, and that the infection can be transmitted by mosquitoes from monkey to monkey as well as from monkey to man, which suggested that monkeys may constitute one of the links in the perpetuation of the virus in nature, Simmons et al , 1931 During World War II, extensive studies resulted in the demonstration of the very small size and other important

Sabin, 1952a). The ultimate adaptation and propagation of both types of dengue virus in very high titer in suckling mice, and the development of complement-fixing and hemagglutinating antigens, permitted further progress in studies on the diagnosis, the epidemiology, and the prevention of dengue (Sabin, 1955, 1956)

CLINICAL PICTURE

The clinical picture presented here is based on manifestations observed in natural cases of the disease and in several hundred human volunteers. The usual incubation period is from 5 to 8 days, although it may vary from 2 5 to 15 days, depending on the amount of virus introduced Prodromal symptoms of headache, backache, fatigue, stiffness, anorexia, chilliness, malaise and occasionally rash may appear 6 to 12 hours before the first rise in temperature In perhaps 50 per cent of the patients the onset is sudden, with a sharp rise in temperature associated with severe headache, pain behind the eyes, backache, pain in the muscles and the joints, chilliness and rarely a shaking chill. In typical cases, the fever (Fig. 53) persists for 5 or 6 days and usually terminates by crisis The temperature rarely exceeds 105° F., only occasionally returns to normal during the middle of the febrile period to give rise to the saddleback or diphasic type of curve, and quite frequently reaches its highest level during the last 24 hours of the febrile period Salicylates affect the fever in dengue and may give rise to very bizarre, spiking temperature curves At first, the pulse rate may rise proportionately to the temperature, but after the first day or two there is usually a relative bradycardia; an absolute bradycardia may occur during convalescence. Anorexia and constipation are common during the entire

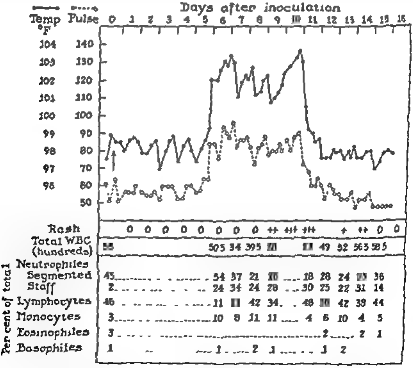

FIG. 53. Graphic representation of temperature and pulse rate of a human volunteer inoculated experimentally with the Hawaiian strain of dengue virus by means of the bites of eight infected *Aedes aegypti* mosquitoes; arrow indicates day on which the patient was bitten Time of appearance of rash is also indicated, as well as total and differential blood counts.

illness. Epigastric discomfort, colicky pain and abdominal tenderness may be seen. Altered taste sensations constitute a very common symptom early in the disease. The patient may become so weak and dizzy that he collapses when he tries to get out of bed. Photophobia, drenching sweats, sore throat, cough, epistaxis, dysuria, hyperesthesia of the skin, pain in the groin and the testicles, and delirium are some of the other manifestations occasionally encountered. A flushed appearance of the face, the neck and the chest, and a punctiform rash, especially over points of friction as at the back of the elbows and the front of the knees, may be seen early in the disease. The bulbar and the palpebral conjunctivae may be injected, the eyes are tender to pressure and painful on movement. Lymph nodes are frequently enlarged, but rarely the spleen. Although occasionally there may be sufficient edema about the fingers to interfere with closure of the hand, large joints present no abnormalities even when there is much subjective pain. Nuchal rigidity is absent even when the patient complains of a stiff neck.

The rash may be maculopapular or scarlatiniform, commonly appears on the third to the fifth day and rarely lasts more than 3 or 4 days. Usually it is seen first on the chest, the trunk and the abdomen and spreads to the extremities and the face. The incidence of this rash varies in different epidemics and also in the experimental disease caused by different strains of virus. Although itching, especially of the palms and the soles, is very common, desquammation occurs only rarely. Another type of eruption, occurring on the last day of fever or shortly after defervescence and consisting of very small petechiae over the dorsum of the feet and the legs, occasionally in the axillae, over the dorsum of the wrists, the hands and the fingers, and on the buccal mucosa and the hard and the soft palates, has been observed in the majority of human volunteers inoculated with the Hawaiian strain of virus (Sabin, 1952a) and in 20 per cent of the cases among American Naval personnel on a South Pacific island (Stewart, 1944). This petechial eruption, unlike the earlier maculopapular or scarlatiniform rash, does not blanch on pressure and fades after 1 to 3 days, leaving a transitory brownish discoloration. So-called Philippine hemorrhagic fever, an illness with extensive petechial rash predominantly on the limbs, epistaxis, gastrointestinal bleeding and shock, recently recognized among Filipino children and young adults, has been associated with infection by dengue virus (Hammon et al., 1958).

Changes in the blood leukocytes are not characteristic for dengue. During the first 24 hours the total number of white cells may be normal, but the lymphocytes show an absolute and relative decrease, while the number of neutrophils is increased by the appearance of immature forms. As the disease progresses, the total number of leukocytes drops, sometimes to as low as 1,500 cells per c.mm., due to a marked diminution in the neutrophils. The lymphocytes begin to increase in number as the neutrophils diminish, and toward the end of the febrile period and early in convalescence frequently constitute the major portion of the circulating leukocytes. The blood picture, as a rule, returns to normal within a week after defervescence. The urine is normal in most cases. The cerebrospinal fluid has been found to be normal in large numbers of patients with dengue, and reports to the contrary cannot be accepted without evidence for the correctness of the diagnosis.

The description given above applies to typical cases, but there is evidence, viz., recovery of virus from patients, that mild febrile illness of from 1 to 3 days' duration without rash may be dengue. Experimental studies on human volunteers have shown that reinfection with a different immunologic type of virus 2 to 3 months after a primary attack may give rise to malaise and slight fever for less than 24 hours, and mosquitoes feeding on such patients acquire the capacity to transmit the infection; furthermore, as the group immunity wears off, infection with a heterologous type of virus has been found frequently to cause febrile illnesses of 2 and 3 days' duration without rash. Since more than one immunologic type of virus has been recovered from the same region at the same time, reinfection with heterologous types of virus may be one of the causes for many of the atypical forms of the disease encountered during certain epidemics (Sabin, 1952a).

Convalescence from severe attacks may take several weeks and is characterized by

marked asthenia. *Occasionally, during* convalescence a disturbance *of vision due to* accommodative weakness or paralysis of the ciliary muscle may occur and persist for from 1 to 4 weeks. Although *there is doubt as to* whether dengue can be a primary cause of death, it has been *so reported during some* of the large *epidemics in* Australia and Greece. The case fatality rate in some *epidemics has* been estimated at 3 per 10,000 (MacCallum and Dwyer, *1927), but* in the recently recognized Philippine hemorrhagic fever, *apparently caused by* dengue virus, the case *fatality rate was 15 per* cent among *750* cases reported in Manila *(Hammon et al., 1958)* A high incidence of spontaneous abortion has *been reported* among women with dengue fever (Magara, 1942).

PATHOLOGIC PICTURE

Goldsmid, 1917, Photakis, *1929*, Catsaras, 1931 and Melissinos, 1937, have *reported on* fatal cases of what they *believed to be* uncomplicated dengue, and described degenerative changes in the liver, the kidneys, the heart or the brain, and hemorrhagic manifestations of varying extent in the endocardium, the pericardium, the pleura, the peritoneum, mucosa of the stomach and the intestines, *muscles*, skin and the central nervous system Local skin lesions caused by the intracutaneous injection of dengue virus, and the maculopapular and petechial eruptions *in human* volunteers were studied by biopsy (Sabin, *1952a*). The epithelium was *not involved*, and no inclusion bodies were found. The *chief* abnormality occurred in and about small blood vessels and consisted of endothelial swelling, perivascular edema and infiltration with mononuclear cells. In the petechial lesions extensive extravasation of blood, without appreciable inflammatory reaction, was observed.

EXPERIMENTAL INFECTION; HOST RANGE

Intracutaneous injection of 0 1 cc. to 0.2 cc. of human serum containing 10 or more minimal *human infective* doses(M I D) of dengue virus is followed after an interval of from 3 to 5 days by local edema and erythema, 1 to 4 cm *in diameter. Serum obtained from experimentally infected* persons within 24 hours after the first rise of temperature was found to contain a million human M.I.D of virus per cc. Ten M.I.D injected intracutaneously produced as severe an infection as *did 1 million M.I.D* Amounts of virus in the range of one M.I D produced: (*1*) typical attacks of dengue; (*2*) mild, short attacks without rash followed by complete immunity, or (*3*) no evidence of infection except a partial immunity. Undiluted infectious serum rubbed on scarified skin produced unmodified dengue Nasal instillation of 1 million or 100,000 M I D. resulted in a very mild disease in some human volunteers, while others suffered from a typical, unmodified attack. Nasal instillation of 10,000 M.I.D. produced neither disease nor immunity. Instillation of 200,000 M.I.D into the conjunctival sac produced typical dengue in one volunteer, while 10,000 M I D. produced neither disease nor immunity in another (Sabin, 1952a) A laboratory worker developed dengue 9 days after human serum containing unmodified dengue virus was accidentally squirted in his eye (Melnick et al , 1948)

Inapparent infection with dengue virus has been produced experimentally in the following species of monkeys: *Cynomolgus fascicularis, Cercopithecus callitrichus, Macacus fuscatus, Macacus philippinensis, Macacus rhesus, Cebus capucinus, Ateles geoffroyi, Ateles fusciceps, Alouatta palliata, Marikina geoffroyi, Saimiri orstedii*, and *Aotus trivirgatus*. Focal infiltrative lesions of the type seen in nonparalytic poliomyelitis have been found in the spinal cord of rhesus monkeys sacrificed 6 weeks after intracerebral injection of human dengue virus; neutralizing and complement-fixing antibodies appeared in these monkeys from 12 to 21 days after inoculation and persisted for many months (Sabin, 1950) An essentially inapparent infection has been produced in chimpanzees (Paul et al., 1948) with development of neutralizing and complement-fixing antibodies which persisted for at least 10 months (Sabin, 1950). *Dogs*, young hogs, rabbits, guinea pigs, adult white mice, white rats, hamsters and cotton rats inoculated with human dengue blood have shown no signs of infection Tests for inapparent infection in dogs, rabbits, young hogs, and guinea pigs have been negative. Suckling or older mice, which fail to develop clinical manifestations

of infection following intracerebral injection of human serum containing dengue virus, are, nevertheless, resistant to infection by the highly virulent mouse-adapted virus during the first days after receiving the former, as a result of interference, and during subsequent months, as a result of active immunity (Sabin, 1950, 1952a).

Several strains of dengue virus have now been adapted to mice and maintained in serial passage by the intracerebral route In the early passages a small and varying proportion of the inoculated mice show clinical evidence of the infection after incubation periods which vary from 5 to 35 days The mice exhibit motor weakness, flaccid paralysis of one or more extremities with or without signs of encephalitis The intracerebral titer may be no more than 10^{-1} to 10^{-2} after the first 10 to 15 passages and 10^{-4} to 10^{-5} after the first 40 passages, during the course of further passages in 2- to 3-week-old mice, the titer rises to a level of 10^{-5} to 10^{-6} with corresponding shortening of the incubation period The titer of virus depends upon the age of the mice in whose brains it is propagated If the mice are from 1 to 7 days old at the time of inoculation, their brains yield virus suspensions with intracerebral titers of 10^{-7} to 10^{-8} per 0 03 cc ($10^{9.5}$ to $10^{9.5}$ LD_{50} per Gm) upon titration in approximately 3-week-old mice However, even after many serial passages in newborn mice, the titer reverts to 10^{-5} to 10^{-6} after a single passage in 14-day-old mice (Sabin, 1950) In the early passages, only young mice were found suitable for intracerebral titrations, but after thorough adaptation of the virus old mice could also be used Histologic examination of the brain and the spinal cord of affected mice indicates that the primary attack is on the neurons By the use of virus that has had only 40 to 50 intracerebral passages, it has not been possible to infect 14-day-old mice by the intraperitoneal route The mouse-adapted dengue viruses tested thus far are not pathogenic for rabbits, guinea pigs, mature young cotton rats and hamsters, although newborn hamsters may be susceptible

Unmodified human virus and early passage levels of mouse-adapted virus inoculated intracerebrally in rhesus or cynomolgus monkeys produce an inapparent infection with only focal infiltrative lesions, chiefly in the brain stem and the spinal cord. Continued passage in mice leads to the emergence of virus particles that are more neurotropic for monkeys, so that intracerebral inoculation of higher passage levels of mouse-adapted virus frequently results in a severe paralytic disease that clinically and histologically may be indistinguishable from poliomyelitis With the type 1 virus (Hawaiian strain), no paralysis was observed in large numbers of monkeys inoculated with amounts varying from 1 to 100,000 mouse LD_{50} of the 15th to the 20th mouse passage virus, while the 118th mouse passage virus produced paralysis more often with smaller doses (5 of 15 monkeys receiving 400 to 40,000 LD_{50}) than with larger doses (1 of 15 receiving 4,000,000 to 40,000,000 LD_{50}) With the type 2 virus (New Guinea C strain) no paralysis was observed among large numbers of monkeys inoculated with 8 to 80,000,000 LD_{50} of the 18th to the 26th passage in mice, but paralysis associated with extensive neuronal lesions in the spinal cord, the brain stem and occasionally also the cerebral cortex occasionally followed inoculation of the same virus after 13 passages in trypsinized monkey kidney tissue cultures (Sabin and Sweet, quoted in Sabin, 1955 and unpublished studies) Neutralizing, complement-fixing and hemagglutinating antibodies develop in all rhesus monkeys inoculated with unmodified or mouse-adapted virus, but the C-F antibodies are of higher titer and persist longer after infection with the unmodified virus

ETIOLOGY

The diameter of the virus, as determined by filtration of highly infectious human serum through gradocol membranes, was found to be from 17 to 25 mμ The virus can be sedimented from human serum by centrifugation at 24,000 r p m for 90 minutes in an 8-inch rotor of a vacuum ultracentrifuge Examination with the electron microscope of preparations, purified by differential centrifugation, from highly infectious human dengue serum revealed dumbbell-shaped structures (700 mμ × 20-40 mμ) which were not found in preparations from normal human serum (Sabin, Schlesinger and Stanley, quoted in Sabin, 1952a) Reagan and Brueckner (1952) found

rods, 42 to 46 mμ in width and 175 to 220 mμ in length, in electronmicroscopic studies of ultracentrifuged suspensions prepared from the brains of mice inoculated with the 114th mouse passage of the same strain (Hawaiian), but not in similar preparations from normal mouse brains On the other hand, Hotta (1953) found only spherical particles measuring about 20 mμ upon electronmicroscopic examination of the mouse-adapted type 1, Mochizuki strain that had been partially purified by adsorption on kaolin and elution with NH_4OH The virus, in the form of human serum, has been preserved in the frozen state at −70° C. and in the lyophilized state at about 5° C. for at least 8 years. Human blood has been found to be infectious after storage in an ordinary refrigerator for several weeks. Dengue virus is very unstable, however, even at refrigerator temperatures in the absence of a suitable stabilizer Rabbit serum, heated at 56° C. for 30 minutes, diluted with an equal amount of saline is used for preparing brain suspensions of the mouse-adapted virus for optimum storage at −60 or −70° C, and 10 per cent heated rabbit serum saline must be used for preparing dilutions of virus for titrations For material that is to be inoculated in human beings, 5 per cent human albumin adjusted to pH 8, has been found suitable for storage, lyophilization and for preparation of dilutions

Dengue virus is readily destroyed by shaking with ether (Hotta and Evans, 1956a). The virus in human serum or in mosquito suspensions is inactivated by ultraviolet light or by 0 05 per cent formalin, but when so inactivated it failed to produce immunity

Earlier reports of cultivation of unmodified dengue virus in chick embryos were not substantiated by adequate proof and have not been confirmed With type 1 (Hawaiian) mouse-adapted virus of high titer at the 18th mouse-passage level, it was possible to obtain serial propagation in chick embryos, but the yield of virus was 1 millionth of that achieved in the brains of suckling mice. The type 2, New Guinea "C" strain (mouse passage 21), despite its high level of multiplication in the brains of suckling mice (10^{-7} to 10^{-8} per 0 03 cc.), completely failed to multiply in chick embryos (Sweet and Sabin, quoted in Sabin, 1955). The best-sustained multiplication in chick embryos was obtained with the type 1 (Hawaiian) virus after 101 passages in mice, but even after 88 egg passages, the yield of virus, which was present mostly in the brain, was still only 1/100 to 1/1,000 of that obtained from the brains of suckling mice (Schlesinger, 1951). The egg-passaged virus retained the increased neurotropism for monkeys exhibited by the 101st mouse passage virus used to initiate the cultures (Sabin, 1955).

Using the early mouse passage levels, it was possible to propagate both types of dengue virus in trypsinized rhesus kidney cultures, but while Sweet and Sabin (quoted in Sabin, 1955) observed no specific cytopathogenic effect, Hotta and Evans (1956b, 1956c) reported late cellular degeneration Extracellular dengue virus is rapidly destroyed at 37° C., and growth curves indicated that the maximum yield of virus was obtainable at 4 days (Sweet and Sabin, unpublished studies) In the early tissue culture passages the type 1 virus yielded not more than $10^{3.8}$ mouse LD_{50} per cc of culture fluid, while the type 2 virus yielded titers of 10^6 LD_{50} per cc; at the 13th tissue culture passage both types of virus yielded 10^6 to 10^7 mouse LD_{50} per cc of culture fluid, but still without cytopathogenic effect

The existence of at least 2 immunologic types of dengue virus was established by (1) active cross-immunity tests in human volunteers, (2) dermal neutralization tests with convalescent sera in human volunteers, (3) neutralization tests in mice with convalescent sera from human beings and rhesus monkeys, and (4) complement-fixation tests with convalescent sera of human beings and rhesus monkeys Human volunteers reinoculated with the same strain of virus proved to be completely immune 18 months after a single experimental infection Since the volunteers lived in nondengue areas, there can be no question of reinforcement of immunity by intercurrent, inapparent infection. The results of reinoculation with a heterologous immunologic type depended on the interval after the original attack During the first 1 to 2 months there was active immunity to heterologous as well as to homologous strains. That this effect is most likely due to a common

antigen and not to nonspecific resistance resulting from a preceding febrile illness was confirmed by the fact that phlebotomus fever convalescents exhibited no such immunity to dengue virus From 2 to 9 months after the initial attack, reinfection with a heterologous type, as a rule, resulted in a modified form of the disease which was of shorter duration, less severe and without rash By this method of comparison 4 of 7 human strains, i e , the Hawaiian and the New Guinea "A" strains, and 2 strains from India belonged to one immunologic type (type 1), while the other 3, New Guinea "B," "C" and "D" belonged to another (type 2) (Sabin, 1950)

Neutralization of mouse-adapted dengue virus by intracerebral tests in mice has been found to be a function of two factors (1) specific antibody which is heat-stable (56° C for 30 min) and (2) a nonspecific, complementlike, heat-labile, accessory substance which produces the maximum inactivation of the sensitized virus after incubation in vitro for 2 hours at 37° C. Thus, when dengue virus with a low mouse intracerebral titer (10^{-3} to 10^{-4}) was used, no neutralization was demonstrable, either when heated serum was used with incubation of the serum-virus mixtures or when fresh or frozen serum was used without incubation of the serum-virus mixtures When both fresh or frozen serum and incubation of the serum-virus mixtures were employed, the neutralization indices of convalescent sera varied with the mouse intracerebral potency of the virus used, indices of 100 to 300 were the rule with early mouse passage viruses having titers of 10^{-3} to 10^{-4}, as compared with 10,000 to 100,000 with virus having an intracerebral titer of about 10^{-7}. With virus of higher potency, the heated dengue antiserum can by itself yield neutralization indices of 100 to 500 However, when an accessory factor is supplied to such a mixture in the form of fresh or frozen, "normal" (i e., no effect on dengue virus per se), undiluted human or guinea pig serum (by preparing the virus dilutions in such serum), the neutralization index becomes 50,000 (Sabin, 1950) While the major neutralizing antibody is type-specific, there is also a group-specific antibody which yields lower titers and occurs in some individuals but not in others This is particularly true of sera from human beings and rhesus monkeys infected with the type 2 dengue viruses, the majority fail to neutralize the type 1 (Hawaiian) virus, while occasional individuals may yield titers of 50 to 500 (Sabin, 1950) Neutralizing antibodies for the Hawaiian type of virus have been found as early as 7 days after onset of fever and for at least 4 years (the longest period tested thus far) after a single experimental infection in human beings in nondengue areas (Sabin, 1950)

Potent complement-fixing antigens (CF) for both types of dengue virus have been prepared from the brains of suckling mice infected with the maximally adapted strains of virus Human beings and monkeys infected with one type of dengue virus develop CF antibodies not only for both types of dengue but frequently also for the West Nile, Japanese B, Murray Valley and yellow fever viruses (Sabin, 1950, 1956) In individuals, who have had no previous infection with the related viruses, the homotypic CF antibody is usually of higher titer than the heterotypic or heterologous antibodies and persists for a longer period Tests on human volunteers residing in areas where none of these viruses is endemic indicated that homotypic CF antibody can persist for at least 4 years after a single infection

Hemagglutinins for chick erythrocytes have been obtained from the brains of suckling mice infected with either type 1 or type 2 dengue viruses by extraction with M/64 borate—KCl buffer, pH 9, in saline Such preparations retain their activity for at least 4 months at 4° C , but are completely inactive after one or more cycles of freezing (dry ice) and thawing The optimum conditions for reaction with the erythrocytes and stability in certain ranges of pH and temperature vary with the type of virus and the number of passages it has had in mice Thus, freshly prepared hemagglutinin from the 19th-mouse-passage, type 2 virus (New Guinea C) with a titer of 1 640 at pH 6 7 at 4° C failed to react at 23° C at pH 6 5 to 6.9, which is optimum for the type 1 virus (Hawaiian) at both 23° C. and 4° C Specific hemagglutination-inhibition (H-I) antibody was readily separated from normal inhibitor in human, monkey and rabbit sera by precipitation with

acetone Dengue antisera, devoid of neutralizing antibody for the heterotypic virus, contain H-I antibody of similar or lower titer not only for the heterotypic dengue virus but also for the West Nile, Japanese B, St. Louis and yellow fever viruses (Sweet and Sabin, 1954a, Sabin, 1956) By means of the H-I test it has been possible to show that at least *15 distinct* viruses, including the 2 types of dengue, form an antigenically related group —the so-called Group B of the arthropod-borne viruses (Casals, 1957, and Chap. 14) The dengue viruses, as well as other members of the Group B viruses, also share two additional characteristics not possessed by other viruses: (1) their multiplication is depressed by a genetically controlled factor present in the PRI strain of white mice (Sabin, 1952b), and (2) they are inactivated by a nonantibody, antiviral factor in human milk (Sabin, 1953)

This group relationship between the dengue and other viruses is not associated with any significant, active cross immunity Human volunteers, immunized with the 17-D strain of yellow fever were not resistant to small amounts of dengue virus, although an interference phenomenon was observed when the interval between administration of these 2 viruses was 7 days or less (Sabin, 1952a) An interference phenomenon between dengue and yellow fever has also been demonstrated in rhesus monkeys and to a certain extent in *Aëdes aegypti* mosquitoes (Sabin and Theiler, quoted by Sabin, 1952a)

DIAGNOSIS

Dengue should be suspected when a disease, having the clinical characteristics described above, occurs in persons living in or having recently arrived from known endemic areas at a time when the specific mosquito vectors are especially prevalent Diagnosis is difficult in sporadic, atypical and primary cases but is relatively easy once the identity of an epidemic has been established Epidemics of febrile illnesses with rash closely simulating dengue can be caused by certain types of ECHO virus, which are ubiquitous, by the West Nile virus (Bernkopf et al, 1953), which is known to occur in the Middle East, certain parts of Africa and Asia, and by leptospira Denguelike illnesses in Africa may be caused by viruses that are not antigenically related to the dengue group of viruses, e.g., the Chikungunya virus first discovered during an epidemic in Tanganyika in 1952-53 (Theiler and Casals, personal communication)

Any attempt at a rapid identification of the causative agent involved in a denguelike epidemic should include intracerebral inoculation of mice with serum or heparinized blood obtained within 24 to 48 hours after onset of fever. West Nile virus is readily recovered in adult mice, and the dengue viruses are detected with great difficulty only in newborn mice. Mice, not older than 2 to 4 days of the so-called Swiss variety, should be inoculated intracerebrally with 0.02 cc. of undiluted blood or serum, and other litters should also receive the 1 10 and 1:100 dilutions in an attempt to circumvent possible interference by large concentrations of virus that is of very low pathogenicity for mice Some strains of dengue virus may paralyze a large number of mice within 9 to 21 days, while others may affect only an occasional mouse or none The brain and the spinal cord of affected mice is passaged intracerebrally in suckling and adult mice and intra-abdominally in suckling mice. A dengue virus may be suspected when only the intracerebrally inoculated suckling mice succumb, and identification must then be undertaken by neutralization with type-specific antisera and also by hemagglutination inhibition A hemagglutinating virus possessing the properties just mentioned, that is inhibited by both types of dengue antisera in the hemagglutination test but is not neutralized by them or only slightly so, may represent a new type of dengue virus When none of the originally inoculated mice succumbs, the presence or the absence of infection with dengue virus can be determined by an intracerebral challenge inoculation with 100 LD_{50} of the mouse-adapted viruses at 4 to 6 weeks (Sabin, 1956). Recently, dengue viruses have been isolated by this procedure directly from the blood of patients in Malaya (Smith, 1956a), Trinidad (Anderson et al., 1956) and the Philippines (Hammon et al, 1958).

Homotypic neutralizing and H-I antibodies appear within 7 days after onset of fever,

while the C-F antibodies appear a week or 2 later (Sweet and Sabin, 1954b) For a presumptive laboratory confirmation of the diagnosis in the individual patient during the course of a dengue epidemic, the H-I test is the test of choice because the convalescent phase serum specimen obtained within a day or two after defervescence already shows a rising titer of H-I antibody by comparison with the acute phase serum It is necessary to use both type 1 and type 2 dengue antigens for this purpose because the heterotypic response may be delayed or of lower titer. In any attempt at rapid identification of the cause of a denguelike epidemic, the H-I test can help only in indicating whether or not a Group B virus is involved, and West Nile virus is the only other member of this group that has produced epidemics of febrile illness with rash The neutralization test is the least affected by group antigenic relationships and can be used for resolving the identity or the type of virus involved in a given case or epidemic (Smith, 1956b)

TREATMENT

There is no specific therapy

EPIDEMIOLOGY

The most important facts in the epidemiology of the disease are that the virus is transmitted only by certain species of *Aedes* mosquitoes, and that human beings, certain spe-

known as *Aëdes hebrideus* (Mackerras, 1946) and *Aedes polynesiensis* Marks (Rosen et al, 1954) are the only proved vectors of the virus. Although circumstances obtaining on Espiritu Santo, New Hebrides, during the epidemic of 1942-1943 (Daggy, 1944) and in certain parts of New Guinea (Mackerras, 1946) strongly pointed to *Aedes hebrideus* as a vector under natural conditions, the opinion has been expressed that "the island form of *Aedes hebrideus* found in the New Hebrides does not appear to be a vector of dengue in nature under normal conditions" (Perry, 1948) *Armigeres obturbans* has been claimed as a vector (Koizumi et al, 1917), but, because the experiments were carried out in Formosa at a time when dengue was occurring, judgment is suspended *Culex fatigans* (*quinquefasciatus*) has been shown to be incapable of acting as a true vector. Experimental studies in the United States (Sabin and Jahnes, quoted in Sabin, 1952a) have

taeniorhynchus, Aëdes cantator, Anopheles punctipennis, Anoph quadrimaculatus and *Culex pipiens*. *Aedes aegypti* mosquitoes can acquire the infection from patients 6 to 18 hours before and for at least 3 days after the onset, Siler et al, 1926 A minimum extrinsic incubation period of 8 days, more usually 11 to 14 days, is required after an infective blood meal before the mosquitoes can transmit the infection, and under suitable conditions of temperature they can function as effective vectors for the rest of their lives, which may vary from 1 to 3 months or more Blanc and Caminopetros (1930) have shown that *Aedes aegypti* may remain infective for at least 174 days Very small numbers of mosquitoes suffice to transmit the infection, the experimental disease having been produced by the bite of 1, Simmons et al, 1931, or 2 mosquitoes, Siler et al, 1926 The virus is not carried from infected mosquitoes to succeeding generations through the eggs It has not been possible to obtain infected mosquitoes by leaving larvae in human serum containing 1,000,000 M I D. of virus per cc. (Sabin, unpublished studies) It is clear, therefore, that the disease is most likely to persist in those areas where the conditions are favorable for the survival of mosquitoes throughout the year, and for this reason Rogers and Megaw (1942) suggested that countries near the equator are probably the permanent reservoir of dengue The newborn human population in these equatorial regions may be enough to keep the cycle of infection going from year to year Although *Aedes aegypti* is strictly domestic in its habits, *Aëdes albopictus* can exist in the bush or the jungle and by keeping the infection going among susceptible monkeys, Simmons

fever

The availability of serologic tests has provided new tools for specific identification of the viruses involved in present and past epi-

demics and in mapping the endemic zones. Thus, the type 1 dengue virus has been incriminated as the predominant virus in the Hawaii epidemic of 1943-44, the extensive Japanese epidemics of 1944-45 (Sabin, 1952a), the epidemic of 1926-27 in Durban, South Africa (Kokernot et al, 1956), an outbreak in Malaya in 1954 (Smith, 1956b), and in the epidemics in Tahiti and other Society Islands of French Oceania in 1944 (Rosen, 1958a) An investigation, carried out in 1954, established that the extensive epidemic which occurred in Panama in 1941-42, was in all probability due to dengue type 2 virus, and that dengue was not endemic in the area and had not been introduced again since persons born after 1942 exhibited no serologic evidence of infection with dengue viruses (Rosen, 1958b) The monkeys inhabiting the forests of Panama have been shown to be susceptible to experimental infection with the dengue viruses (Rosen, 1958c), but there was no evidence of natural infection among them, and together with the very low incidence of dengue neutralizing antibodies among people residing close to the forests, there is no evidence for a jungle reservoir of type 1 or 2 dengue in Panama (Rosen, 1958b) On the other hand, dengue seems to continue to be endemic in Trinidad (Downs et al, 1956), and a dengue virus antigenically closely related to type 2 was recovered from the blood of a patient in 1953 (Anderson et al, 1956) *Aëdes aegypti* appears to be the only vector in both urban and rural areas, but the extent to which it is currently infested with certain types of dengue is unknown, since only 1 of 109 children under 10 years of age had neutralizing antibodies for type 1 dengue compared with an incidence ranging from 25 to 85 per cent among older individuals (Downs et al, 1956) Rosen (1958b) has indicated that available evidence strongly suggests that in the Western Hemisphere *Aedes aegypti* is the only vector for dengue and that the current campaign for eradication of this mosquito may lead to the total elimination of dengue from this part of the world

Serologic surveys in Malaya and Borneo (Smithburn, 1954; Sabin, 1955; Smith, 1956c) and in India (Smithburn et al, 1954) indicated that both type 1 and type 2 dengue viruses are highly endemic in these parts of Asia, although type 1 seems to be more prevalent. Smith (1958c) found neutralizing antibodies for type 1 dengue virus in about 25 per cent of Malayans below the age of 11 years, in 50 per cent aged 11 to 20, and in almost 100 per cent over 30 years of age; a certain number of persons had neutralizing antibodies only for type 2 dengue, but the precise incidence of infection with this virus was difficult to determine. During World War II, both type 1 and type 2 dengue viruses were active at the same time in New Guinea (Sabin, 1952a) Although Africa has long been regarded as an endemic area for dengue on clinical and epidemiologic grounds, there is as yet no specific virologic evidence of persistent dissemination of dengue viruses anywhere in Africa

In contrast with the negative findings in Panama, evidence of infection with dengue or with a closely related virus was found among mainly tree-living animals in Malaya (28 of 40 monkeys, 7 of 12 slow lorises, 9 of 45 squirrels, 2 of 28 civets) but in very few of a large number of ground-dwelling forest animals (Smith, 1958c) Smith (1958c), therefore, postulated (1) a possible dengue reservoir among tree-dwelling animals maintained by some mosquito or mosquitoes not biting at ground level; (2) *Aedes albopictus*, which is present in large numbers in the forest fringe and biting chiefly outdoors serving as the link between the forest and the rural dengue in man, and (3) *Aedes aegypti* as the main vector responsible for urban outbreaks.

CONTROL MEASURES

To prevent the recurrence of epidemics, it is necessary to maintain a constant vigil against the breeding of *Aedes aegypti* mosquitoes In regions like Hawaii where, in addition to *Aedes aegypti*, *Aedes albopictus* mosquitoes multiply unchecked outside of cities, the control of mosquito breeding presents an especially difficult problem (Usinger, 1944). It takes fewer mosquitoes to keep a dengue epidemic going than are required for the continued dissemination of yellow fever (Hanson, 1936). Antimosquito measures on airplanes and ships contribute to prevention but they cannot keep out a person who may be in the incubation period of dengue. In the face of an epidemic, individual measures directed against mosquito breeding in all sorts of containers in the immediate vicinity of homes, offices and factories, as well as the judicious use of DDT residual spray inside these buildings, must receive the greatest attention, while mass spraying from airplanes

may be useful in the attack on *Aëdes albopictus* breeding in the outskirts of the human community

The possibility of preventing dengue by vaccination first appeared when it was found that the type 1, Hawaiian strain, beginning with the 7th passage in mice, lost its capacity to produce the severe illness and protracted fever characteristic of the natural disease in human beings but retained the capacity to produce a rash and solid immunity to the unmodified virus (Sabin and Schlesinger, 1945) Blood taken at the time the rash first appeared in persons receiving the modified virus, and inoculated into susceptible human volunteers produced only the modified infection *Aedes aegypti* mosquitoes feeding on people inoculated with the 10th-mouse-passage virus acquired the capacity to transmit the infection but only in the modified form Extensive tests carried out on people inoculated with the 15th-mouse-passage virus indicated that *Aëdes aegypti* mosquitoes, which had fed daily on the experimental subjects for a period of 14 days after inoculation, were unable to transmit the infection even after prolonged extrinsic incubation periods Thirty-three human volunteers inoculated with vaccine prepared from early passages of the type 1 virus in 2- to 3-week old mice in doses ranging from 1 to not more than 100 mouse PD_{50} or LD_{50}, developed maculopapular eruptions of varying extent, while systemic symptoms were either absent or very mild, and all were found to be immune upon exposure to *Aedes aegypti* mosquitoes of proved infectivity or inoculation of large doses of unmodified dengue virus An attempt to prepare a combined dengue and yellow fever vaccine by lyophilizing a mixture of the two viruses was unsuccessful, since 5 of 7 volunteers subsequently exposed to dengue-infected mosquitoes were not immune (Sabin, 1952a)

After strains of the type 2 dengue virus had been propagated in high titer in the brains of suckling mice, tests in human beings with both the New Guinea "C" (Sabin, 1955) and New Guinea "B" strains (Schlesinger et al, 1956) in doses of 10^4 to $10^{6.4}$ mouse LD_{50} indicated that the mutant strains behaved like the type 1 modified virus Further work on the practical aspects of vaccination against dengue with the modified type 1 and type 2 dengue viruses established the following significant points (Sabin and Sweet, unpublished studies):

1. Only early passage level strains should be used, since variants that are more neurotropic for monkeys (although not more so than the 17D yellow fever virus) appear on continued passage in mice
2. The vaccine should be prepared from the brains of suckling mice, because: (A) there is either little or no multiplication in chick embryos, and (B) both types of virus (and especially type 2) after thorough adaptation to monkey kidney cultures were more neurotropic for monkeys than the mouse-passaged seed virus
3 The optimum diluent for lyophilization of vaccine is 5 per cent human albumin adjusted to pH 8 0
4 The administration of 10^4 or more mouse LD_{50} of the type 1 virus produces a higher incidence of transitory malaise and occasionally low febrile reactions for a period of 2 to 4 days
5. The simultaneous administration of equal amounts (about 10^4 mouse LD_{50}) of both types of dengue virus led to a suppression or marked delay of the type 1 antibody response in some individuals
6. For administration of the smaller doses that need to be given to avoid the higher incidence of febrile reactions and malaise, the lyophilized vaccine must be diluted in 5 per cent human albumin, adjusted to ph 8, and maintained at ordinary refrigerator temperature (not more than a few hours) up to time of inoculation; otherwise, the virus quickly deteriorates, and no immune response is obtained
7 Both types of vaccine administered separately produced enough heterotypic cross immunity for a period of at least 6 weeks to prevent the appearance of rash or other signs, when type 1 vaccine was given 6 weeks after the type 2, no type 1 antibody appeared, but when type 2 vaccine was given 6 weeks after type 1, antibody development was delayed but not prevented
8. The H-I antibody provides a quick, simple and reliable measure of the response to vaccination

The experimental studies carried out thus far suggest that the following procedure of vaccination may be attempted in the case of need in the face of an extensive dengue epi-

demic or for the protection of those moving into highly endemic areas:

1 Inoculate type 1 vaccine only in a dose of about 10^3 suckling mouse LD_{50}, this would be expected to produce some sort of rash, grading from minimal to extensive, [redacted] almost all, and some transitory slight muscle aching and soreness behind the eyes, usually relieved by acetylsalicylic acid, in about 60 per cent after an incubation period of 10 to 16 days This may be expected to provide not only long-lasting immunity to type 1 dengue, which is the most common cause of large epidemics, but also a transitory protection against the type 2 virus for a period of about 8 weeks.

2. Depending on circumstances, the type 2 vaccine, in a dose not exceeding 10^4 suckling mouse LD_{50}, can be administered next but not earlier than 8 to 10 weeks after the type 1.

In the face of an epidemic definitely known to be caused predominantly by a type 2 dengue virus, it would of course be advisable to use the type 2 vaccine first

REFERENCES

(Other references, particularly those of historic interest, may be found in the second edition, Chapter 33)

Anderson, C R , Downs, W G , and Hill, A E , 1956, Isolation of dengue virus from a human being in Trinidad, Science *124, 224-225*

Bernkopf, H , Levine, [redacted] , and Nerson, R , 1953, Isolation of West Nile virus in Israel, J Infect Dis *93*, 207-218

Blanc, G , and Caminopetros, J , 1930, Recherches expérimentales sur la dengue, Ann Inst Pasteur *44, 367-436*

Casals, J , 1957, The arthropod-borne group of animal viruses, Tr New York Acad Sc Ser *2, 19*, 219-235

Daggy, R H , 1944, *Aedes scutellaris hebrideus*, probable vector of dengue in the New Hebrides, War Med *5, 292-293*

Downs, W G , Anderson, C R , and Theiler, M , 1956, Neutralizing antibodies against certain viruses [redacted] the sera of residents of Trinidad, B W I , Am J Trop Med & Hyg *5*, 626-641

Hammon, W. McD , Rudnick, A , Sather, G E , Rogers, K D , La Motte, L C , Jr , Chan, V , and Dizon, J J , 1958, Philippine hemorrhagic fever, etiological relationship to dengue, Am J Trop Med & Hyg , in press

Hanson, H , 1936, Some observations on dengue, Am J. Trop Med *16, 371-375*

Hotta, S , 1953, Partial purification of the mouse-adapted dengue virus, Acta scholae med univ Kioto *31, 7-10*

Hotta, S , and Evans, C A , 1956a, Ether-sensitivity of dengue virus, Virology *2, 704-706*

———, 1956b, Cultivation of mouse-adapted dengue virus (type 1) in rhesus monkey kidney tissue culture, J Infect Dis *98, 88-97*

———, 1956c, Cultivation of type 2 dengue virus in rhesus kidney tissue culture, Proc Soc Exper Biol & Med *93*, 153-155

Koizumi, T , Yamaguchi, K , and Tonomura, K , 1917, An epidemiological study of dengue fever, J Med Assn Formosa (nos 176-177), Abstr in Trop Dis Bull , 1918, *12, 77*

Kokernot, R H , Smithburn, K C , and Weinbren, M P , 1956, Neutralizing antibodies to arthropod-borne viruses in human beings and animals in the Union of South Africa, J Immunol *77*, 313-323

Lumley, G F , and Taylor, F. H , 1943, Dengue, [illegible] Health

[illegible]

dengue up until 1943)

McCallum, F , and Dwyer, J P , 1927, Dengue as a cause of death, M J Australia *1*, 10-15

Mackerras, I M , 1946, Transmission of dengue fever by *Aëdes* (Stegomyia) *scutellaris*, Walk. [redacted] New Guinea Tr Roy. Soc Trop Med & Hyg *40*, 295-312

Magara, M , 1942, Dengue fever in the fields of obstetrics and gynecology, Nippon Igaku, No 3306, *2286* (In Japanese)

Melnick, J L , Curnen, E C , and Sabin, A B , 1948, Accidental laboratory infection with human dengue virus, Proc Soc Exper Biol & Med *68*, 198-200

Paul, J. R , Melnick, J L , and Sabin, A B , 1948, Experimental attempts to transmit phlebotomus (sandfly, pappataci) and dengue fevers to chimpanzees, Proc. Soc Exper Biol & Med *68*, 193-198

Perry, W J , 1948, The dengue vector on New Caledonia, the New Hebrides, and the Solomon Islands, Am J Trop Med *28*, 253-259

Reagan, [redacted] L , and Brueckner, A L , 1952, Studies on dengue fever virus by electron microscopy, J Bact *64*, 233-235

Rogers, L , and Megaw, J W D , 1942, Tropical Medicine, ed 4, pp *156*, London, Churchill

Rosen, L , 1958a, Dengue antibodies in residents of the Society Islands, French Oceania, Am J Trop Med & Hyg *7*, 403-405

———, 1958b, Observations on the epidemiology of dengue in Panama, Am. J Hyg. *68*, 45-58

———, 1958c, Experimental infection of new world monkeys with dengue and yellow fever viruses, Am J Trop Med & Hyg *7*, 406-410

Rosen, L , Rozeboom, L. E , Sweet, [redacted] H , and Sabin, A B , 1954, The transmission of dengue by *Aëdes polynesiensis* Marks, Am J Trop Med & Hyg *3*, 878-882

Sabin, A B , 1950, The dengue group of viruses and its family relationships, Bact Rev *14*, 225-232

Sabin, A B, 1952a, Research on dengue during World War II, Am J Trop Med & Hyg 1, 30-50
———, 1952b, Nature of inherited resistance to viruses affecting the nervous system, Proc Nat Acad Sc 38, 540-546
———, 1953, Relationships between arthropod-borne viruses based on antigenic analysis, growth requirements, and selective biochemical inactivation, Ann New York Acad Sc 56, 580-582
———, 1955, Recent advances in our knowledge of dengue and sandfly fever, Am J Trop Med & Hyg 4, 198-207
———, 1956, Dengue in Diagnostic Procedures for Virus and Rickettsial Diseases, ed 2, pp 383-394, New York, Am Pub Health Ass'n
Sabin, A B, and Schlesinger, R W, 1945, Production of immunity to dengue with virus modified by propagation in mice, Science 101, 640-642
Schlesinger, R W, 1951, Propagation in chick embryos of dengue virus, Hawaiian strain II Findings in infected eggs, Proc Soc Exper Biol & Med 76, 817-823
Schlesinger, R W, Gordon, I, Frankel, J W, Winter, J W, Patterson, P R, and Dorrance, W R, 1956, Clinical and serologic response of man to immunization with attenuated dengue and yellow fever viruses, J Immunol 77, 352-364
Smith, C E G, 1956a, Isolation of three strains of type 1 dengue virus from a local outbreak of the disease in Malaya, J Hyg 54, 569-580
———, 1956b, A localized outbreak of dengue fever in Kuala Lumpur epidemiological and clinical aspects, M J Malaya 10, 289-303
———, 1956c, The history of dengue in tropical Asia and its probable relationship to the mosquito *Aëdes aegypti*, J Trop Med & Hyg 59, 243-251
Smithburn, K C, 1954, Neutralizing antibodies against arthropod-borne viruses in the sera of long-time residents of Malaya and Borneo, Am J Hyg 59, 157-163
Smithburn, K C, Kerr, J A, and Gatne, P B, 1954, Neutralizing antibodies against certain viruses in the sera of residents of India, J Immunol 72, 248-257
Stewart, F H, 1944, Dengue analysis of the clinical syndrome at a South Pacific advance base, U S Nav M Bull 42, 1233-1240
Sweet, B H, and Sabin, A B, 1954a, Properties and antigenic relationships of hemagglutinins associated with the dengue viruses, J Immunol 73, 363-373
———, 1954b, Development of different antibodies in human beings experimentally infected with type 1 or type 2 dengue viruses, Fed Proc 13, 513-514
Usinger, R L, 1944, Entomological phases of the recent dengue epidemic in Honolulu, Pub Health Rep 59, 423-430

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17

Phlebotomus Fever

(SYNONYMS Sandfly fever, pappataci fever, 3-day fever, mosquito fever)

INTRODUCTION

Phlebotomus fever is a self-limited, nonfatal, phlebotomus-transmitted illness of virus etiology, characterized by fever, severe headache, pain in the eyes, conjunctival injection, malaise and leukopenia The term "sandfly fever," popularly used among English-speaking people, has not been accepted in international scientific literature because biting insects belonging to the genus *Culicoides* and having no relation to the disease under discussion are also called sandflies Pappataci fever, the popular name for the disease in Italy and the Balkans, has also failed to find general acceptance The name "phlebotomus fever," first suggested by Newstead, 1911, and stressing the most important fact about the disease, namely, its specific relationship to the insects of the genus *Phlebotomus,* has been used by many writers and seems to be the most appropriate for international usage.

HISTORY

Although Pick, *1886,* is generally credited with the first description of the disease as a clinical entity under the name of Hundskrankheit (dog disease—*apparently because* the conjunctival injection was so striking that the eyes resembled those of a bloodhound), Birt, *1913,* and Whittingham, *1924,* call attention to Sir William Burnett's account of Mediterranean fever during the Napoleonic wars in 1799, Pym's description of Bulam fever in Gibraltar in 1804, and the reports of other British military surgeons during the early *19th century* on *Malta summer febricula*

between the midges known as the pappataci flies (*Phlebotomus papatasi,* Scopoli) and the disease from which newly arrived Austrian troops suffered each summer, and led to the *classic experiments of the Austrian* military commission consisting of Doerr, Franz and Taussig, 1909. By appropriate tests on human volunteers living in areas free from the dis-

... that the etiology

the vector Being a constant problem to foreign troops stationed in endemic areas, the disease has been the subject of study by several British military commissions; Whittingham and Rook, *1923;* Young et al, *1926;* Shortt et al., *1935;* and during World War II *for the first time by American investigators* (Sabin et al, *1944;* Sabin, *1951;* Hertig and Fisher, *1945*).

More recent studies have resulted in the adaptation of at least *2* immunologically *distinct types of the virus (Sicilian and Naples)* to suckling and adult ...

quent development of neutralization, complement-fixation and hemagglutination-inhibition tests and the demonstration that the modified viruses had lost their capacity to produce disease in man but were still immunogenic (Sabin, 1955).

CLINICAL PICTURE

Observations on more than 100 cases of the experimental disease in human beings produced with different strains of virus are the basis for this description of the clinical manifestations of the malady The incubation period of 3 or 4 days in the average case may vary from 2 5 to [illegible] days, exceptional incubation periods, one each of 50 hours, 7 days and 9 days, have been seen, following intravenous inoculation, however, incubation periods of 42 to 44 hours are common The onset of the disease is sudden in the majority of cases In some there may be a prodromal period characterized by malaise, giddiness, constipation and abdominal distress The following signs and symptoms may be encountered at the onset or sometime during the course of the disease, although some of them are frequently absent in otherwise typical cases: frontal headache, burning sensation or pain in eyes, photophobia, backache, stiffness in neck and back, pains in joints and extremities, anorexia, nausea, vomiting, abdominal distress, alteration or loss of taste, constipation during the first few days and diarrhea during convalescence, sore throat,

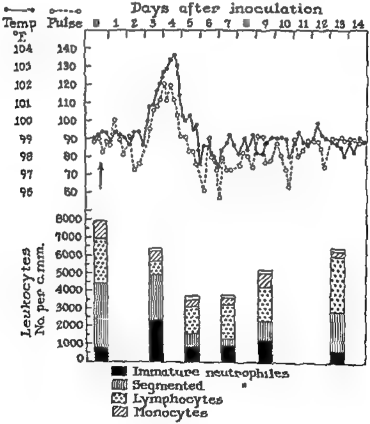

FIG 54 Graphic representation of temperature, pulse rate and blood picture of a human volunteer experimentally inoculated with the virus of phlebotomus fever, arrow indicates time of inoculation

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the popular belief that there was a connection between the midges known as the pappataci flies (*Phlebotomus papatasi*, Scopoli) and the disease from which newly arrived Austrian troops suffered each summer, and led to the classic experiments of the Austrian military commission consisting of Doerr, Franz and Taussig, 1909. By appropriate tests on human volunteers living in areas free from the disease, the commission proved that the etiology [illegible]

the vector. Being a constant problem to foreign troops stationed in endemic areas, the disease has been the subject of study by several British military commissions, Whittingham and Rook, 1923; Young et al., 1926, Shortt et al., 1935; and during World War II for the first time by American investigators (Sabin et al., 1944, Sabin, 1951; Hertig and Fisher, 1945).

More recent studies have resulted in the adaptation of at least 2 immunologically distinct types of the virus (Sicilian and Naples) to suckling and adult mice, with the conse-

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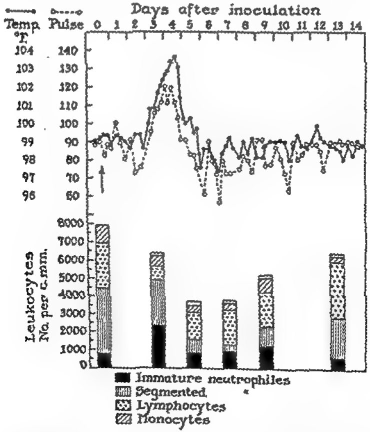

FIG 54 Graphic representation of temperature, pulse rate and blood picture of a human volunteer experimentally inoculated with the virus of phlebotomus fever, arrow indicates time of inoculation

epistaxis, chills or chilliness, profuse sweating, giddiness and weakness, especially during convalescence

The temperature usually reaches its peak, not over 104 5° F, within 24 or 48 hours after onset, and the febrile period in 85 per cent of the cases is 2, 3 or 4 days (Fig 54) Fevers of less than 1 day's duration and of 5 to 9 days' duration may occur The pulse rate is usually elevated in proportion to the fever on the first day of the disease, and thereafter returns to normal more rapidly than does the temperature A bradycardia may be present at the end of the febrile period and during convalescence Other physical findings are limited to an erythema of the face and exposed parts of the neck and the chest, conjunctival injection which is occasionally limited to the exposed portion of the ocular conjunctiva (Pick's sign), tenderness of the eyeballs, and congestion of the fauces, the soft palate and the posterior pharyngeal wall Rhinitis, tracheobronchitis, lymphadenopathy, and enlargement of the liver and the spleen are not a part of the disease Urticaria or erythema multiforme may be encountered occasionally, but a true rash as seen in dengue does not occur

Changes in the leukocytes are no different from those seen in dengue, infectious hepatitis and certain other infections with leukopenia. The characteristic finding on the first day of the fever is a total count within normal limits, a relative and absolute decrease in the lymphocytes accompanied by a relative and sometimes absolute increase in the neutrophils which is due to an increase in immature cells During the second or third day of fever, the number of lymphocytes begins to return to normal and for a few days thereafter may constitute from 40 to 65 per cent of the total count At the same time, the number of neutrophils begins to drop, and the immature cells increase to a point at which they usually outnumber the segmented cells The greatest drop in the total number of leukocytes may not occur until the end of the febrile period or after defervescence, but the marked shift to the left in the neutrophils and changes in the proportions of different types of cell at various stages of the disease are more important for diagnosis than is a single total leukocyte count. The urine is normal The cephalin-cholesterol-flocculation and phosphatase tests for hepatic damage are negative. The cerebrospinal fluid is normal in experimental cases, and there is no evidence that the pleocytosis reported in some outbreaks (Fleming et al, 1947) can be caused by the virus of phlebotomus fever.

The duration of convalescence varies with the individual and the climate; marked uncontrollable, transitory mental depression is encountered occasionally. Recurrences of the fever and symptoms during convalescence from the naturally acquired disease have been reported by several investigators and have also been observed from 5 to 7 days after the initial defervescence among 5 per cent of the experimentally inoculated volunteers (Sabin et al, 1944)

PATHOLOGIC PICTURE

There have been no fatalities among thousands of uncomplicated cases, and nothing is known of the pathologic changes produced by the virus.

EXPERIMENTAL INFECTION; HOST RANGE

No evidence of pathogenicity has been obtained with virus of proved infectivity for human beings after inoculation in the following lower animals by the intracerebral, intracutaneous, subcutaneous, intratesticular intranasal or intraperitoneal routes: young baboons (*Papio hamadryas*), grivet (*Cercopithecus griseoviridis*), vervet (*Cercopithecus aethiopis centralis*), red African hussar (*Cercopithecus patas*), *Macaca radiata* and *Macaca mulatta* (rhesus monkeys), 2- to 3-week old white mice, wild gray mice, cotton rats Egyptian desert rats (jerboas), Syrian hamsters, guinea pigs and rabbits The virus could not be demonstrated in the serum of rhesus monkeys 3 and 4 days after inoculation by subinoculation in human beings of proved susceptibility (Sabin et al, 1944) Six chimpanzees inoculated with human serum believed to contain the virus exhibited no clinical signs of infection. A slight febrile response in the 2 chimpanzees whose temperatures were being recorded could not be interpreted with certainty (Paul et al., 1948).

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Work carried out since 1952 resulted in the adaptation to mice of the two known immunologically distinct types of virus, represented by the Sicilian and the Naples strains. The Sicilian strain in the form of infectious human serum had been stored in the frozen state for 9 years before it was successfully adapted to the brains of newborn mice. Three blind passages in 2- to 4-day-old mice at 7-day intervals yielded a virus that produced encephalitis after an incubation period of 11 to 12 days. After 10 passages in newborn mice at a time when the titer was already $10^{-3.4}$ per 0.01 cc., the virus still produced no clinical manifestations in weaned mice. Continued passage in the brains of newborn mice finally yielded a virus that produced encephalitis in newborn as well as weaned mice at a titer of 10^{-7} per 0.01 cc., i.e., 10^9 LD_{50} per Gm. of brain from infected newborn mice. Intracerebral inoculation of the highly pathogenic mouse-adapted strain produced a fatal encephalitis in 5- to 6-day-old rats, but neither clinical signs nor lesions in baby hamsters (18 to 20 Gm.), guinea pigs, rabbits or rhesus monkeys, since antibody appeared after this single inoculation, there may have been an inapparent infection in these animals. The mouse-adapted virus also lost its capacity to produce illness in human beings but still produced antibody and resistance to infection with the unmodified human virus.

A mouse-pathogenic variant of the immunologically distinct Naples strain was obtained from infected human serum after a single blind, intracerebral passage in newborn mice. The initial incubation period of 14 days was reduced to 8 days after 5 consecutive intracerebral passages in 2- to 4-day-old mice but despite a titer of $10^{-3.3}$ per 0.01 cc. it still produced no signs in 4-week-old mice. It was not until 35 consecutive intracerebral passages in newborn mice that the virus became regularly pathogenic for weaned mice, and in its 55th passage it yielded similar titers (still only $10^{-3.4}$ to 10^{-4} per 0.01 cc.) in newborn and weaned mice, although the incubation period was longer in the older mice. This fully adapted virus produced clinical manifestations in suckling and older mice also after nasal instillation but not after subcutaneous or intra-abdominal inoculation. Neither clinical signs nor lesions were observed in intracerebrally inoculated young rabbits, guinea pigs and baby hamsters, but neutralizing antibodies developed in the guinea pigs and the hamsters. Rhesus monkeys inoculated intracerebrally with either 10^5 or 10^4 mouse LD_{50} of the 30th passage virus all exhibited fever for about 10 days after an incubation period of 8 to 16 days, but no signs of paralysis, weakness or encephalitis. Histologically, these monkeys exhibited foci of interstitial infiltration and

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perivascular cuffing in the posterior and lateral horns of the spinal cord, medulla, midbrain, thalamus, hypothalamus, and only occasionally and minimally in the parietal and temporal cortex Despite the febrile episodes and lesions, only 1 of 9 monkeys developed a significant level of neutralizing antibodies Four human beings, inoculated intracutaneously with $10^{3.0}$ mouse LD_{50} of the 30th passage virus, exhibited neither fever nor clinical manifestations, and all developed neutralizing antibodies When these individuals were subsequently inoculated with unmodified human Naples virus that produced a moderately severe febrile illness in 2 simultaneously inoculated control individuals, 2 were entirely resistant, and 2 had a slight, transitory febrile response with about 10^2 mouse infective doses per cc. of serum at the time of the fever, which is 200 to 10,000 times less than was found in the serum of the controls (Sabin and Sweet, quoted in Sabin, 1955, also unpublished studies)

Ananyan (1953) reported that pappataci fever virus from certain areas in the U S S R multiplied in the blood, the lungs and the brain of young mice, guinea pigs, rats and rabbits either inapparently or with the production of "the clinical picture of the disease" The conclusions were based largely on the demonstration of complement-fixing activity of uncertain specificity in serum, lungs or brain of the inoculated animals. One of the strains that had been submitted to many passages in the brains of young mice (about 5 Gm), followed by many passages in the allantoic cavity of chick embryos, was used as a live vaccine against pappataci fever in the U.S S R. This vaccine was submitted by Dr. Ananyan for comparative studies, but it was neither pathogenic for newborn mice nor did it induce in them any immunity to challenge with the mouse-adapted Sicilian and Naples strains (Sabin, unpublished studies). Soshnikova (1954) reported the adaptation of pappataci fever viruses by intracerebral inoculation of 1- to 15-day-old mice with the production of a disease in mice that "follows the usual clinical course of pappataci fever" The relation of the pappataci fever viruses reported by investigators in the U.S.S R to those described here is as yet uncertain.

ETIOLOGY

By passing infectious human serum through gradocol membranes it was found that filtrates from membranes with an average pore diameter (A.P.D.) of about 200 mμ or more regularly produced the disease in human volunteers, while filtrates from membranes with an A.P.D. of 100 mμ or less did not (Sabin et al, 1944; Sabin, 1951). From these findings it was estimated that the diameter of the virus is not greater than 40 or 60 mμ but it could be smaller, since the concentration of virus in the human serum used for filtration was relatively low.

Recent studies with mouse-adapted viruses of higher potency indicated that the size of the virus is closer to 17 to 25 mμ. When a filtrate containing $10^{6.6}$ mouse LD_{50} per cc. of the Sicilian strain was passed through gradocol membranes of progressively smaller A P D., the virus passed through membranes with an A P.D. of 79 mμ but not through those with an A P.D of 50 mμ or less However, when only about 10^5 mouse LD_{50} per cc. were present in the suspension used for filtration, the virus failed to pass membranes with an A P.D. of 98 mμ. The Naples strain yielded the same filtration endpoints as the Sicilian strain when equivalent amounts of virus were filtered (Sabin and Sweet, unpublished studies).

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Multiple attacks of clinically similar illnesses during the same season or in subsequent seasons have led many to doubt that one attack of phlebotomus fever produces appreciable immunity. However, experiments on human volunteers residing in phlebotomus-free regions of the United States have shown that a solid immunity to reinfection with the same strain of virus is present as long as 2 years (longest period tested thus far) after a single experimental attack of the disease (Sabin, 1951) Three different strains of phlebotomus fever virus were obtained by human passage from natural cases occurring during 1943 and 1944 in troops stationed in the Mediterranean area (Sabin, 1951) Two of these, one from the Middle East and one from Sicily, proved to be identical by cross-immunity tests carried out 1 month, 4 months and 2 years, respectively, after primary attacks The third strain, recovered during an outbreak in Naples, possessed the characteristic properties of phlebotomus fever virus but was completely different immunologically from the other two Human volunteers proved to be immune to reinfection with the homologous Naples virus, developed typical, unmodified attacks of the disease after inoculation of the Sicilian virus within 2 months of the first infection Likewise, those who recovered from infection with the Sicilian virus suffered from unmodified attacks of the disease after inoculation with the Naples virus (Sabin, 1951) This complete lack of cross-immunity is unlike the picture exhibited by the multiple immunologic types of dengue virus in which a group relationship is manifested by cross-immunity tests during the first 1 or 2 months after the initial attack and by modified forms of the disease at later periods The Naples strain of virus, which is thus a completely distinct immunologic entity, has not yet been submitted to the ultimate test for a phlebotomus fever virus, namely, transmission by *Phlebotomus papatasi*

Both the Sicilian and the Naples mouse-adapted viruses propagated in the brains of newborn mice yielded complement-fixing (C-F) antigens of high potency which react specifically and in high titer with the respective hyperimmune sera prepared in mice. No C-F antibodies have been detected in the convalescent sera of human beings experimentally infected with the unmodified Sicilian strain, and titers of only 1 2 have been found with the Naples virus antigen in convalescent sera of those experimentally infected with the unmodified Naples virus

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perivascular cuffing in the posterior and lateral horns of the spinal cord, medulla, midbrain, thalamus, hypothalamus, and only occasionally and minimally in the parietal and temporal cortex Despite the febrile episodes and lesions, only 1 of 9 monkeys developed a significant level of neutralizing antibodies Four human beings, inoculated intracutaneously with $10^{5.9}$ mouse LD_{50} of the 30th passage virus, exhibited neither fever nor clinical manifestations, and all developed neutralizing antibodies When these individuals were subsequently inoculated with unmodified human Naples virus that produced a moderately severe febrile illness in 2 simultaneously inoculated control individuals, 2 were entirely resistant, and 2 had a slight, transitory febrile response with about 10^2 mouse infective doses per cc of serum at the time of the fever, which is 200 to 10,000 times less than was found in the serum of the controls (Sabin and Sweet, quoted in Sabin, 1955, also unpublished studies).

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DIAGNOSIS

The diagnosis of phlebotomus fever is generally made on clinical and epidemiologic grounds The disease is suspected when outbreaks of an illness with fever of short duration occur during the hot, dry season, especially among immigrants, tourists, or foreign troops in countries known to harbor *Phlebotomus papatasi* Differentiation from outbreaks of dengue is aided by the shorter duration of fever and the absence of rash and lymphadenopathy in phlebotomus fever Influenza is not common during the hot season and can be diagnosed by serologic reactions In localities where malaria is also prevalent and when, because of the pressure of events, antimalarial therapy may be administered in the absence of a positive smear, thousands of cases of phlebotomus fever have been erroneously diagnosed as malaria, Birt, 1915 (Sabin et al, 1944). Infectious hepatitis at the onset may simulate phlebotomus fever, but the subsequent appearance of jaundice or a positive cephalin-cholesterol test serves to differentiate the two conditions. In certain parts of Africa, yellow fever and Rift Valley fever may cause confusion, but their true identity can be established by recovery of the viruses of these diseases from patients and by neutralization tests. The clinical manifestations resulting from infection with many of the enteroviruses (ECHO, Coxsackie and polioviruses) may be most difficult to differentiate from those of phlebotomus fever.

Specific proof of infection with phlebotomus fever viruses can be obtained only by isolation of virus, which is now possible in suckling mice, and by the demonstration of development of antibody against the known types during convalescence. By intracerebral inoculation of 1- to 3-day-old mice with serum obtained early after onset of fever in suspected cases, more than 20 strains of phlebotomus fever virus were isolated in recent years in Egypt. The majority of these strains were immunologically very closely related or identical with the Sicilian type, and a few strains with the Naples type of virus C-F antibodies against the newly isolated as well as against the prototype strains were apparently more readily demonstrated in the sera of the naturally infected patients (R M Taylor, personal communication). Neutralizing antibodies may be demonstrated within 2 weeks after onset of fever. An indirect way of demonstrating the presence of one of the established types of virus in human serum consists of inoculating 1- to 3-day-old mice intracerebrally with 0 01 cc of serum and 4 weeks later challenging one group by an intracerebral inoculation of 100 LD_{50} of the Sicilian virus and another group with the Naples virus

TREATMENT

No specific therapy is available.

EPIDEMIOLOGY

According to present knowledge the disease is maintained in nature by passage from man to man through the medium of the intermediate host and vector, *Phlebotomus papatasi* Secondary cases do not arise by contact in the absence of the vector Although other species of *Phlebotomus*, e g, *perniciosus* and *caucasicus*, have been found in areas where

the disease has occurred, there is no experimental proof that they may act as vectors

Phlebotomus papatasi is a sand-colored, hairy midge, 2 to 3 mm long, somewhat less than 1 mm thick, easily recognized by the position of its 2 wings which are elevated and spread to form a V. The body of the female appears distended and red for some hours after a blood meal, and black for several days thereafter. Only the female bites and usually does so during the night and early hours of the morning. The site of a bite may or may not be marked by a reddish, pinpoint spot, and before a person becomes sensitive to the insects there is neither pain nor irritation beyond that of the initial stab However, from 1 to 2 weeks after the primary exposure to such bites, markedly inflamed, itching papules (2 to 3 mm in diameter, pink or red, sometimes vesicular) usually appear at almost all of the sites originally bitten and may persist for 4 or 5 days Once sensitization is established, such papules appear soon after bites The flies are most prevalent near the ground level, and, because of their small size and ability to squeeze through small apertures, ordinary screens and mosquito nets fail to exclude them Their flight is conducted as a series of short hops, alighting on stones and other obstacles in their approach to a house, and then, instead of entering at once, they tend to alight on the outer walls where they continue to hop about with relatively long pauses between hops After entering a house they continue to hop about on the walls and ceiling for some time before attempting to feed These peculiar habits of phlebotomus flies render them especially vulnerable to the residual action of DDT (Hertig and Fisher, 1945) Breeding places of the insects are difficult to demonstrate, but typical sites are found in loose soil, organic debris beneath stones, cracks in masonry, embankments, rubble, and dark, protected spots containing sufficient moist organic matter (Whittingham and Rook, 1923) The larvae are not aquatic and are killed by too much moisture They thrive during hot, dry seasons, and under optimum conditions 4 5 to 6 weeks are required for the eggs to develop into winged insects The life of the adult is relatively short in hot weather, and in the laboratory it lives no longer than 2 or 3 weeks An extrinsic incubation period of 7 to 10 days after feeding on a patient is known to be sufficient for transmission, but not enough work has been done to be certain that a shorter period will not suffice.

The geographic distribution of the disease is regarded as being limited to the areas harboring *Phlebotomus papatasi*, which include particularly those parts of Europe, Africa and Asia that lie between 20° and 45° north latitude. The disease is definitely known to occur in Sicily, Italy as far north as the Po Valley, along the Adriatic coast of Yugoslavia, Greece, Malta, Crete, Cyprus, Egypt, Israel, Jordan, Syria, Iraq, Iran, the coast of Crimea, the Azov and Black Sea littoral, provinces of central Asia in the U S S R, and the northwest and central provinces of India The disease is not known to occur in the United States, although man-biting species of *Phlebotomus, P diabolicus* in Texas and an undetermined species in the Okefenokee Swamp, Georgia (Johannsen, 1943), have been reported Whether or not these and other man-biting species of *Phlebotomus* in the western hemisphere are capable of acting as vectors of phlebotomus fever virus is not known. *Aëdes aegypti, Culex pipiens, Pulex irritans* (Sabin et al, 1944; Sabin, 1951) and bedbugs, Doerr et al, 1909, have been tested and found incapable of transmitting the infection.

Phlebotomus fever, unlike dengue, is not known to have invaded new territories. Large outbreaks have invariably occurred among troops or immigrants from countries free from the malady who had moved into endemic regions. Although the total number of cases of phlebotomus fever officially reported as hospital admissions in the U S Army during World War II was only 12,434, the actual number was undoubtedly much greater, thousands of cases having been reported as fever of unknown origin or as malaria (Sabin et al, 1944). Although the disease is practically never recognized among the native populations of endemic regions, it is obvious that the infection is acquired in infancy and childhood While it is clear how new susceptible subjects become available each year to aid in the perpetuation of the virus, it is not clear what the reservoir of the virus is during the late autumn and winter months when the phlebotomus flies are absent. The virus quickly disappears from the blood of human beings, and there is no evidence that it may be carried by lower animals Doerr and Russ, 1909, first suggested the possibility that the virus may be transmitted from one generation of infected *Phlebotomus papatasi* to another Whittingham, 1924, reported that *Phlebotomus papatasi* reared in the laboratory in England produced the disease in human be-

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Moshkovsky, S D, Diomina, N A, Nossina, V. D, Pavlova, E A, Livshitz, J L, Pels, H J, and Roubtzova, V P, 1937, Pappataci fever VIII On the transmission of the virus of pappataci fever in phlebotomi born from eggs laid by infected females, Med Parasitol 6, 922-937 (in Russian)

Paul, J R, Melnick, J L, and Sabin, A B, 1948, Experimental attempts to transmit phlebotomus (sandfly fever, pappataci) and dengue fevers to chimpanzees, Proc Soc. Exper Biol & Med 68, 193-198

Philip, C B, Paul, J R, and Sabin, A B, 1944, Dimethyl phthalate in control of phlebotomus (pappataci or sandfly) fever, War Medicine 6, 27-33

Sabin, A B, 1951, Experimental studies on phlebotomus (pappataci, sandfly) fever during World War II, Arch f die gesamte Virusforschung 4, 367-410

———, 1955, Recent advances in our knowledge of dengue and sandfly fever, Am J. Trop Med & Hyg 4, 198-207

Sabin, A B, Philip, C B, and Paul, J R, 1944, Phlebotomus (pappataci or sandfly) fever: a disease of military importance, summary of existing knowledge and preliminary report of original investigations, J A M A 125, 603-606, 693-699

Soshnikova, M N, 1954, Studies on the virus of mosquito [Phlebotomus] fever in suckling mice, Zhur Mikrobiol Epidemiol, i Immunol, No 7, 53-55 (in Russian)

Whittingham, H E, and Rook, A F, 1923, Observations on the life history and bionomics of *Phlebotomus papatasii*, Brit M. J 2, 1144-1151

ings without previously feeding on infected persons, however, he believed that the larvae might have acquired the virus by feeding on the dejecta or dead bodies of adult flies Others, Young et al, 1926, noting that the flies did not die in their breeding grounds after oviposition and that the larvae showed no preference for feeding on the dead bodies of adult flies, maintained that infection of the insect probably occurred in their breeding grounds and suggested that the mites (*Trombidium hindustanicum* Hirst and *Raphignathus youngi* Hirst) which were found on about 4 per cent of adult phlebotomus flies might constitute the true reservoir of the virus Moshkovsky et al (1937), starting with the ova of thousands of flies which had been fed on patients, proved in a series of well-controlled experiments that certain of the adults raised from ova hatched away from their "parents" were capable of producing phlebotomus fever in human volunteers, that a virus caused the illness was established by serial passage in other human beings. The American commission (Sabin et al, 1944) was unable to produce the disease with offspring from flies of proved infectious capacity or from larvae which had ingested lyophilized virus, and concluded that transmission of the virus from one generation of flies to another is not a regular event The question of how the virus overwinters and what its true reservoir may be cannot be regarded as having been settled.

CONTROL MEASURES

Control measures are directed against the vector. DDT residual spray is effective against *Phlebotomus papatasi* (Hertig and Fisher, 1945) and should be used to control the vector in living quarters and breeding sites within a radius of 100 to 200 meters, which is the usual flight range for flies breeding near human habitations. Regular use of dimethyl phthalate and other effective insect repellents after sundown and upon retiring [illegible] the dis- [illegible] 944) [illegible] epidemics (Cullinan and Whittaker, 1943) were not infrequent in certain types of military installations, and separate wards, located from 300 to 600 meters away from other buildings, were considered desirable for patients with this disease.

Many reports have appeared in recent years of the success attained in the eradication of pappataci fever in various endemic foci of the disease in the U.S.S.R As a result largely of the systematic application of DDT and hexachlorocyclohexane in 1948 to 1952, the breeding of Phlebotomus was markedly reduced in Simferopol and the incidence of the disease in 1952 was believed to be about 800 times less in 1952 than in 1947 (Korolev et al, 1954). In 1956, the disease was regarded as being practically eradicated in Crimea and Ashkhabad, and vaccination was no longer used as a means of controlling pappataci fever in the U.S.S.R (V. M Zhdanov, personal communication). The actual virus content and the effectiveness of the live virus vaccine formerly used in the U.S.S.R. (Ananyan, 1953) is difficult to evaluate. While the mouse-adapted Sicilian and Naples strains possess properties that might make them useful as live virus vaccines, the problem of the practical development of such vaccines has not been pursued, chiefly because of the great effectiveness of DDT and related compounds in control of the vector.

REFERENCES

(Other references, particularly those of historic interest, may be found in the second edition, Chapter 34)

Ananyan, S A, 1953, The problem of active immunization against mosquito [Phlebotomus] fever Part I Adaptation of the mosquito fever virus to laboratory animals Part II Specific prophylaxis of pappataci fever with live vaccine, Zhur Mikrobiol, Epidemiol, i Immunol, No 1, 32-40 (in Russian)

Cullinan, E R, and Whittaker, S R F, 1943, Outbreak of sandfly fever in two general hospitals in the Middle East, Brit M J 2, 543-545

Fleming, J, Bignall, J R, and Blades, A N, 1947, Sandfly fever review of 664 cases, Lancet 1, 443-445

Hertig, M, and Fisher, A A, 1945, Control of sandflies with DDT, Bull U S Army Med Dept No 88 (May) 97-101

Johannsen, O A, 1943, A noxious species of Phlebotomus in the Okefenokee Swamp, Georgia, Psyche 50, 112-113

Korolev, P A, Markov, T L, and Tikhomirova, N I, 1954, Result of eradication of an endemic focus of pappataci fever, Med Paraz i Paraz Bol, No 4, 344. (In Russian, the same issue contains other reports on eradication of pappataci fever elsewhere in the U S S R)

severe aching of the muscles of the back and the legs are the prominent symptoms of onset; anorexia, nausea, vomiting, photophobia, muscle pains and hyperesthesia of the skin may be a part of the syndrome The temperature rises rapidly and is sustained from 102° to 104° F for about 2 days The pulse rate is increased correspondingly Usually on the third day a period of remission occurs that lasts 2 or 3 days During this time the temperature will be normal or even subnormal, but the patient is quite weak—exceptionally so for such a short illness The remission is followed by a relapse of 2 to 3 days The temperature may rise somewhat higher than in the first phase, and the febrile period may last a day or so longer Lloyd (1951) claims that well over 90 per cent of all cases show "this typical saddleback type of temperature curve" Meiklejohn (1957) has noted that while the great majority of cases follow the well-documented course of a "saddleback" or a "double hump" of fever, yet a number of exceptions do occur, and monophasic temperature curves are not too rare Occasionally, triple bouts of fever have been reported by others In addition, Meiklejohn states that he has accumulated considerable evidence of the occurrence of subclinical infections which were demonstrated only by neutralization tests on blood-serum samples

The white blood cell count falls to 2,000 or 3,000 cells per cu mm with the lowest count usually being found during relapse There is no exanthem with the disease, and the general physical examination is essentially negative except for the febrile condition and slight injection of the throat and the conjunctivae Following the recrudescence, patients frequently complain of great weakness which may last for several weeks The prostration is quite similar to that seen following influenza or dengue

No patients have been reported to have had the disease twice, and Florio et al (1944) showed experimentally that one attack of the disease confers a high degree of immunity to human volunteers Lloyd (1951) notes that on two occasions he has seen "punched-out ulcers" to form at the site of tick-bite. These are difficult to treat and apparently resemble the type of ulcer seen in scrub typhus Becker likewise noted that indolent ulcers occur frequently at the site of tick-bite and stated that they are not characteristic of ordinary secondary infection. Recently, Eklund et al (1955) have called attention to the fact that Colorado tick fever is not always the relatively mild or benign disease that the literature suggests Thus, virus was isolated from the blood of 5 children, all of whom showed signs of central nervous system involvement Encephalitis was suspected in 4 of the patients, and meningitis in the 5th One child was described as comatose, and 2 as disoriented and delirious More recently, Eklund (1957) reported that a young child, with evidence of encephalitis and a bleeding tendency, and from whom the virus of Colorado tick fever was isolated, died

Meiklejohn (1957) likewise has observed central nervous system involvement in 3 cases of Colorado tick fever. Of these 3, 2 showed a clinical picture which suggested an encephalitis similar to that seen with other viruses such as western equine encephalomyelitis or St Louis encephalitis The third case clinically resembled any of the comparatively benign viral meningitides with no real evidence of involvement of the brain itself This patient simply showed a stiff neck and back and a mild pleocytosis in the spinal fluid Present indications are that such cases of Colorado tick fever with involvement of the central nervous system are not too common However, it is seen from the above that the disease is not always mild.

PATHOLOGIC PICTURE

Nothing is known of the pathologic picture in man. In hamsters the only constant and significant lesions are found in the spleen (Black et al, 1947) Sections of spleens from infected animals stained with hematoxylin and eosin show alterations in the cellular type and arrangement of the follicular lymphoid tissue, with variations in the extent but not in the type of reaction Apparently, there is a reduction in the number of lymphoid cells in the central [illegible]

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18

Colorado Tick Fever

(SYNONYMS Mountain fever, tick fever, mountain tick fever, nonexanthematous tick fever, American mountain fever)

INTRODUCTION

Colorado tick fever is a nonexanthematous disease of man, characterized by a short-course and intermittent fever, and is the only tick-transmitted virus disease of man that is recognized in the Western Hemisphere.

HISTORY

Colorado tick fever has been described ever since white immigrants settled the Rocky Mountain region and undoubtedly was mentioned over a 100 years ago in medical reports of Army doctors stationed at various camps and forts in the Rocky Mountain area. Waggoner in 1850 described a series of typhoid fever cases among which were certain mild forms—possibly mountain fever Hygiene reports of the United States Army by Patzki, Towne, and Jaquette in 1875 contained many accounts of mild variants of malarial, typhoid or possibly undulant fever which fit the description of mountain fever. Toomey, 1931, reviewed these early reports and proposed the name *American mountain tick fever* for the disease However, Becker, 1930, was the first to report the condition as a separate clinical entity, to describe the disease clearly, to raise the question of the tick as a possible etiologic agent and to suggest the name it now bears, *Colorado tick fever*. In 1940, Topping et al, while conducting a clinical

that the disease is associated with the bite of the wood tick, *Dermacentor andersoni*. Florio et al. (1944) transmitted the disease serially in man and hamsters by parenteral injection of blood or serum and showed that the virus passes collodion filters. In subsequent papers they reported isolation of the virus from naturally infected ticks, *D. andersoni*, collected near Denver (1950a), and from *D. variabilis*, collected on Long Island, N. Y. (1950b). Koprowski and Cox (1946, 1947a, 1947b) adapted the virus to the mouse and the developing chick embryo. They showed that the virus was not related to the commonly known mosquito- or tick-transmitted viruses. Oliphant and Tibbs (1950) found 3- or 4-day-old Swiss albino mice suitable for isolating virus and reported the first isolations of Colorado tick fever viruses from patients who had been exposed outside of Colorado and Wyoming

CLINICAL PICTURE

From 4 to 5 days after exposure to ticks there is a sharp and clearly defined onset of the disease, and it is not unusual for a patient to be able to establish the exact time of onset The disease usually starts with chilly sensations and severe aching of the entire body. Fever, headache, deep ocular pain and

severe aching of the muscles of the back and the legs are the prominent symptoms of onset; anorexia, nausea, vomiting, photophobia, muscle pains and hyperesthesia of the skin may be a part of the syndrome The temperature rises rapidly and is sustained from 102° to 104° F for about 2 days The pulse rate is increased correspondingly Usually on the third day a period of remission occurs that lasts 2 or 3 days During this time the temperature will be normal or even subnormal, but the patient is quite weak—exceptionally so for such a short illness The remission is followed by a relapse of 2 to 3 days The temperature may rise somewhat higher than in the first phase, and the febrile period may last a day or so longer. Lloyd (1951) claims that well over 90 per cent of all cases show "this typical saddleback type of temperature curve" Meiklejohn (1957) has noted that while the great majority of cases follow the well-documented course of a "saddleback" or a "double hump" of fever, yet a number of exceptions do occur, and monophasic temperature curves are not too rare Occasionally, triple bouts of fever have been reported by others In addition, Meiklejohn states that he has accumulated considerable evidence of the occurrence of subclinical infections which were demonstrated only by neutralization tests on blood-serum samples

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ragged border of mononuclear cells, with occasional polymorphonuclear leukocytes and erythrocytes Giemsa stain shows both eosinophilic and basophilic intracytoplasmic inclusions present in the large mononuclear cells.

EXPERIMENTAL INFECTION, HOST RANGE

In the early passages, hamsters inoculated intraperitoneally with infectious blood remained normal in appearance, but starting with the 12th passage, some began to die, and thereafter a mortality rate of 25 to 50 per cent was common. Serial passage of the virus by parenteral injection of blood or serum through a series of 6 human volunteers did not alter the virulence of the virus for hamsters, although the incubation period in man was shortened to 3 days instead of 4 to 6 (Florio et al, 1946a) Dilute brown agouti mice or albino Swiss mice inoculated intracerebrally with mouse-adapted virus first show signs of paralysis of the legs, soon become prostrate and die, generally on the 5th to the 7th day. Mice 8 days of age are just as susceptible to intraperitoneal infection as are 28-day-old mice to infection induced by intracerebral inoculation. Resistance to virus inoculated intraperitoneally begins to be evident in 14-day-old mice Infected mouse-brain tissue is lethal for mice by the intracerebral route in 10^{-5} and 10^{-6} dilutions. Following intracerebral inoculation, mice, cotton rats, hamsters and opossums show virus in their blood, but sheep and rabbits do not; only the mice and the hamsters die of viral infection initiated by this route (Koprowski and Cox, 1947a). The virus grows in chick embryo tissue when the yolk sac method of inoculation is used The virus, which is found chiefly in the central nervous tissue of the chick embryo, reaches its maximum titer ($10^{3.3}$ to $10^{4.0}$ LD_{50}) in 4 or 5 days. Chick-embryo-propagated virus retains its virulence for mice and hamsters inoculated by the intracerebral route

Oliphant and Tibbs (1950) reported the primary isolation of 10 strains of Colorado tick fever virus by intraperitoneal inoculation of albino Swiss mice, 3 to 5 days old, using either blood serum or emulsified blood-clot from human beings suspected of having the disease and living in Colorado, Wyoming, Oregon or Utah. In 5 instances, signs of illness were seen in the first-passage mice within 5 to 8 days following inoculation Isolation of the other 5 strains required blind-passage technic in which infected brain tissue was transferred by intraperitoneal injection on the 5th day. All 10 strains were immunologically similar to the Florio strain first adapted to mice in 1946 by Koprowski and Cox. Great difficulties were experienced in adapting the established virus strains to young adult Swiss mice, but this was accomplished by using in successive passages mice that were 16 to 17 days old and 17 to 18

ETIOLOGY

The virus readily passes Berkefeld N and W candles and single Seitz EK pads By the use of collodion membranes, the diameter of the hamster-adapted virus was estimated to be 10 mμ (Florio, et al, 1946b), while the diameter of the mouse-brain-adapted virus was estimated to be 35 to 50 mμ (Koprowski and Cox, 1947a). The virus, when present in serum, is particularly stable, surviving for at least 3½ years in the ice compartment of an ordinary refrigerator or in a commercial deep-freeze unit. It is readily preserved by freezing and drying, may be inactivated by heating at 60° C for 30 minutes and apparently is relatively unstable in tick suspensions (Eklund et al, 1955).

The virus introduced in minimal amounts parenterally elicits an immune response in mice so that they can withstand subsequent intracerebral challenge with massive doses of mouse-brain virus (Koprowski and Cox, 1947b)

By cross-immunity tests in human volunteers, Florio et al (1946c) and Pollard et al (1946) showed that the viruses of Colorado tick fever and dengue are not related Neutralization tests carried out in mice (Koprowski and Cox, 1946; 1947a), or complement-fixation tests (De Boer et al, 1947) show that the virus of Colorado tick fever is different from the viruses of Russian spring-summer encephalitis, louping-ill, Venezuelan equine encephalitis, western equine encephalitis, eastern equine encephalitis, Japanese B encephalitis, St Louis encephalitis, lymphocytic choriomeningitis, rabies, yellow fever

and dengue fever, and also distinct from the rickettsiae of murine (endemic) typhus, Rocky Mountain spotted fever and Q fever.

DIAGNOSIS

A history of exposure to ticks, especially to *D. andersoni*, the biphasic or saddleback temperature curve, the symptomatology and leukopenia suggest the diagnosis of Colorado tick fever Neither acute phase nor convalescent sera show any significant Weil-Felix reaction in the presence of Proteus OX-19, OX-2, or OX-K antigens Specific complement-fixing antigens have been prepared from infected mouse brains, they do not give false positive reactions in the presence of positive-syphilitic sera (De Boer et al, 1947) A fairly close correlation is obtained in the complement-fixation and mouse-neutralization tests with human convalescent sera According to Eklund (1957) the neutralization test is a more useful diagnostic test in Colorado tick fever than in most virus diseases because antibodies tend to appear rather slowly Antibodies have been found absent as long as 26 days after onset of illness in cases where virus was isolated, in some cases only low antibody titers have been seen much later, but more often both complement-fixing and neutralizing antibodies do appear in good titer within 2 weeks They may remain demonstrable for at least 34 months

TREATMENT

There is no specific treatment Treatment is symptomatic

EPIDEMIOLOGY

As mentioned above, Colorado tick fever is the only tick-transmitted virus disease of man that is known in the Western Hemisphere Patients invariably give a history of having been in a tick-infested area 4 or 5 days previous to the onset of illness and in most cases have found a tick or ticks attached to their bodies The disease is most prevalent at the peak of the tick season The ticks are present in the late spring and early summer when the ground and the covering vegetation are moist Early cases may be seen in March and continue to increase to a peak in May and June Relatively few cases occur in July and later

wood tick, *Dermacentor andersoni*, the only species incriminated thus far in transmission of the disease to man. A possible exception to the above was noted by Eklund (1957) who stated that a virus isolation was made from *D occidentalis* ticks collected slightly outside the *D andersoni* area in Oregon The virus has also been isolated from other species of ticks collected within the *D andersoni* habitat area, such as *D parumapertus*, *D occidentalis*, *Otobius lagophilus* and *D. albipictus*, but no evidence exists as yet that any of these ticks are concerned in the transmission of Colorado tick fever to man (Eklund et al, 1955, Kohls, 1955; Philip and Hughes, 1953 and Philip et al, 1955) It is worthy of note, however, that neutralizing antibodies for Col-

macentor tick *D parumapertus* infests man, yet it is known that this tick, as well as *D andersoni*, infests blacktailed jackrabbits in some areas where the ranges of these two ticks overlap. Eventually this information may prove to be of more than academic interest (Philip et al, 1955)

Figure 55 (furnished by Dr Carl M Eklund, The Rocky Mountain Laboratory, U S. Public Health Service, Hamilton, Mont) shows the distribution of *D andersoni* and *D parumapertus* and the area in which Colorado tick fever has been isolated from human patients Virus has been isolated from lots of *D andersoni* collected in every state within its distribution with the exception of those collected in North Dakota, Nebraska and Arizona (Eklund, 1957) However, so few ticks have been examined from these states that the negative results are of no significance Virus has been isolated from ticks collected in several areas in western Washington, so that it is quite likely that the comparative absence of Colorado tick fever cases there may be due to lack of recognition of the disease Virus has also been isolated from ticks collected in Banff National Park, Alberta, Canada (Banfield, 1956), but no evidence of the disease has been reported there as yet Eklund et al (1955) stated that through the

year 1954 they had isolated Colorado tick fever virus from the blood of 193 patients exposed as follows California, 1, Colorado, 38, Idaho, 52, Montana, 16, Nevada, 34; Oregon, 26, Utah, 6; Washington, 1; and Wyoming, 19.

As would be expected, the majority of persons affected are those whose activities bring them in contact with ticks, such as cattlemen, foresters, sheepmen, fishermen and tourists

Florio et al (1950a) reported that the virus is transmitted transovarially by the adult female *D andersoni* They demonstrated transmitted virus in the larval, nymphal and subsequent adult life-cycle stages, although they were unable to detect virus in the eggs of the originally infected adults The same authors (1950b) also reported the isolation of 3 strains of Colorado tick fever virus from dog ticks, *Dermacentor variabilis*, collected on Long Island, N. Y.; hamsters were used in this work. Thus far, no human cases of Colorado tick fever have been reported from this area Pertaining to these questions, Eklund has recently pointed out (1957) that thus far he and his associates have secured no evidence of transovarial transmission of virus in *D andersoni* They believe that the virus is maintained by a cycle between immature ticks and small rodents. Furthermore, they have never isolated Colorado tick fever virus from *D variabilis*, although they have examined thousands of such ticks collected in both the eastern and the western United States Eklund et al (1955) have also attempted an estimate of the incidence of virus-carrying ticks in cer-

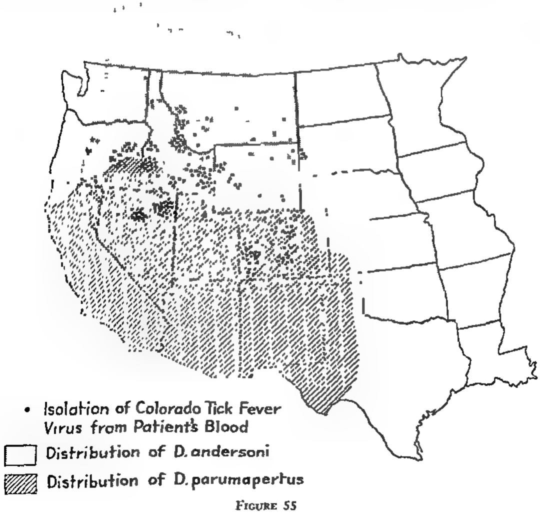

FIGURE 55

tain heavily infected areas of the Bitter Root valley of western Montana; the Boulder, Colorado, area and the Elko, Nevada, area In the Bitter Root valley, the incidence of virus-carrying ticks was estimated to vary from 1 3 to 10 3 per cent, in the Boulder area from 3.7 to 14 3 per cent or greater, and in the Elko area from zero to 6 5 per cent

Using the 3-day-old suckling-mouse technic of Oliphant and Tibbs (1950), Eklund (1957) has isolated at least 336 Colorado tick fever virus strains from patients' blood mailed in from various parts of the West during the past few years The following is the distribution of the isolations from blood samples prior to 1953, 63, 1953, 46, 1954, 87, 1955, 76 and 1956, 64 In addition, several hundred isolations were made from ticks collected in various parts of the West The increase in size of the area in which Colorado tick fever has been recognized does not reflect an increased effort to detect the disease in the field but rather increased attempts at the isolation of virus in the laboratory (Eklund [redacted] al, 1955) Philip and Hughes (1953) state that new laboratory technics may disclose that Colorado tick fever is the most widespread tick-borne disease of man in the western United States

CONTROL MEASURES

The prevention of Colorado tick fever depends primarily upon the avoidance of tick-infested areas and the early removal of any ticks that may become attached to the body It is also recommended that suitable clothing (high boots, leggings, or socks worn outside the trouser legs) be worn so that ticks will find it more difficult to become attached The arthropod repellants, dibutyl-phthalate and dimethyl-phthalate, have been suggested as being useful for impregnation of clothing to prevent the attachment of ticks (Joint OIHP/WHO Study-Group on African Rickettsioses, 1950) Koprowski and Cox (1947b) and Koprowski et al (1950) reported on the vaccination of a total of 24 human volunteers with active, chick-embryo-adapted virus Three of the 24 individuals probably were immune prior to vaccination and these showed no clinical reaction whatsoever Twenty showed enlarged, tender axillary lymph nodes adjacent to the site of inoculation, which appeared on the 6th or 7th day after injection and subsided within 24 to 48 hours. Twelve had a mild attack of illness which lasted from 2 to 5 days and resembled Colorado tick fever, except that there was only a single febrile response, and the onset and the disappearance of symptoms were more gradual than is usually seen in the natural disease One individual had circulating virus on the 6th and the 8th days after inoculation, and 2 others on the 6th day. All vaccinated individuals, including the 3 presumably immune, developed neutralizing antibodies The latter showed an earlier and greater serologic response Later, 5 of the inoculated individuals were challenged with a living, human strain of Colorado tick fever virus and apparently were immune. Thus far no practical applications of these findings have been made available

REFERENCES

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Banfield, A W F, 1956, An investigation of ticks as disease vectors in Banff National Park, Alberta, Canad J Zool 34, 417-423

Black, W C, Florio, L, and Stewart, M O, 1947, A histologic study of the reaction in the hamster spleen produced by the virus of Colorado tick fever, Am J Path 23, 217-225

De Boer, C J, Kunz, L J, Koprowski, H., and Cox, H R., 1947, Specific complement-fixing diagnostic antigens for Colorado tick fever, Proc Soc Exper Biol & Med 64, 202-208

Eklund, C M, 1957, Personal communication Rocky Mountain Laboratory, U S Public Health Service, Hamilton, Montana

Eklund, C M., Kohls, G M, and Brennan, J M, 1955, Distribution of Colorado tick fever and virus-carrying ticks, J.A.M.A 157, 335-337

Florio, L., Hammon, W McD., Laurent, A., and Stewart, M O, 1946c, Colorado tick fever and [illegible]

Dermacentor andersoni in nature and a laboratory study of the transmission of the virus in the tick, J Immunol 64, 257-263

———, 1950b, Colorado tick fever Isolations of virus from *Dermacentor variabilis* obtained from Long Island, New York, with immunological comparisons between eastern and western strains J Immunol 64, 265-272

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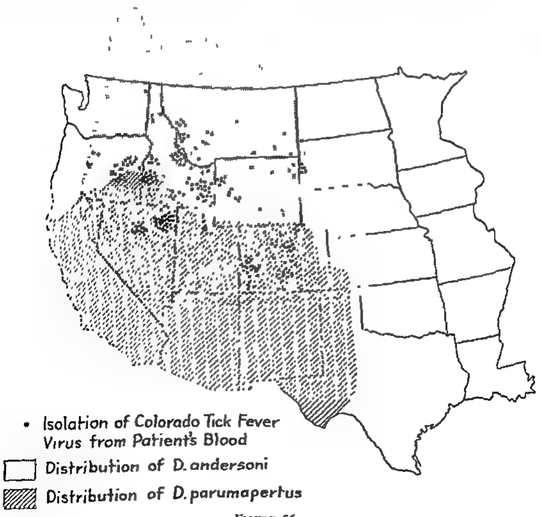

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REFERENCES

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Banfield, A W F., 1956, An investigation of ticks as disease vectors in Banff National Park, Alberta, Canad. J Zool 34, 417-423

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De Boer, C J, Kunz, L J, Koprowski, H, and Cox, H R, 1947, Specific complement-fixing diagnostic antigens for Colorado tick fever, Proc Soc Exper Biol & Med 64, 202-208

Eklund, C M, 1957, Personal communication Rocky Mountain Laboratory, U S Public Health Service, Hamilton, Montana

Eklund, C M, Kohls, G M, and Brennan, J M, 1955, Distribution of Colorado tick fever and virus-carrying ticks, J A M A 157, 335-337

Florio, L, Hammon, W McD, Laurent, A, and Stewart, M O, 1946c, Colorado tick fever and Dengue An experimental immunological and clinical comparison J Exper Med 83, 295-301

Florio, L, Miller, M S, and Mugrage, E R, 1950a, Colorado tick fever Isolation of the virus from *Dermacentor andersoni* in nature and a laboratory study of the transmission of the virus in the tick, J Immunol 64, 257-263

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Florio, L, Mugrage, E R, and Stewart, M O, 1946a, Colorado tick fever, Ann Int Med 25, 466-472

Florio, L, Stewart, M O, and Mugrage, E R, 1944,

The experimental transmission of Colorado tick fever, J Exper Med 80, 165-188

Florio, L, Stewart, M O, and Mugrage, E R, 1946b, The etiology of Colorado tick fever, J Exper Med *83*, 1-10

Joint OIHP/WHO Study-group on African Rickettsioses Report on the first session. World Health Organization Technical Report Series No 23. World Health Organization, Geneva, December 1950

Kohls, G M, 1955, Colorado tick fever discovered in California California Vector Views, *2*, April Published by the Bureau of Vector Control, State Department of Public Health, Berkeley, Calif

Koprowski, H, and Cox, H R, *1946*, Adaptation of Colorado tick fever virus to mouse and developing chick embryo, Proc Soc Exper Biol & Med *62, 320-322*

———, *1947a*, Colorado tick fever *I* Studies on mouse brain adapted virus J *Immunol 57, 239-253*

———, *1947b*, Colorado tick fever *II* Studies on chick embryo adapted virus J *Immunol 57, 255-262*.

Koprowski, H, Cox, H R, Miller, M S, and Florio, L, 1950, Response of Man to egg-adapted Colorado tick fever virus, Proc. Soc Exper Biol. & Med *74*, 126-131.

Lloyd, L W, *1951*, Colorado tick fever, M Clin *North America 35, 587-592*

Meiklejohn, G, 1957, Personal communication, Dept of Medicine, University of Colorado Medical Center, Denver, Colorado

Oliphant, J W, and Tibbs, R. O., 1950, Colorado tick fever Isolation of virus strains by inoculation of suckling mice Pub Health Rep *65, 521-522*

Philip, C B, Bell, J F, and Larson, C. L., 1955, Evidence of infectious diseases and parasites in a peak population of black-tailed jack rabbits in Nevada J. Wildlife Management *19(2)*·*225-233*

Philip, C B, and Hughes, L E, 1953, Disease agents found in the rabbit tick, *Dermacentor parumapertus*, in the southwestern United States In 6th International Congress of Microbiology, Rome, Sept 6-12 (Sect 16), *5*, 541-548

Pollard, M, Livesay, H R, Wilson, D J, and Woodland, J. C, *1946*, Immunological studies of dengue fever and Colorado tick fever. Proc Soc. *Exper Biol. & Med 61, 396-398*

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19

Miscellaneous Arthropod-Borne Virus Infections of Man

Although many of the arthropod-borne viruses can be classified in the A or B Groups, a considerable number show no such immunologic relationship The present chapter deals with viruses falling in this category which have been studied sufficiently well to justify their inclusion Other viruses which fall outside the A and B Groups are those causing phlebotomus fever and Colorado Tick fever (Chaps. 17 and 18)

RIFT VALLEY FEVER

(Synonym Enzootic Hepatitis)

Introduction

Rift Valley fever is an acute disease principally of sheep and cattle and is caused by a specific virus In man the disease is characterized by a short incubation period, acute onset, fever of several days' duration, prostration, pain in the extremities and the joints, abdominal discomfort and leukopenia The mortality rate is low, and the immunity following recovery is lasting Man is usually infected secondarily during the course of an epizootic in domesticated animals The natural disease has been reported only from Africa

History

During an extensive epizootic of an hitherto undescribed disease in sheep in the Rift Valley in East Africa in 1930, Daubney et al (1931) isolated a virus. Workers associated with these investigations contracted a short but severe febrile disease, and a similar clinical picture was common among the herders of the infected flocks The natural disease in sheep was ill-defined, consisting of listlessness, disinclination for food, and prostration The mortality was high in newborn lambs, death being due to acute liver necrosis, pregnant ewes aborted. Daubney et al (1931) showed that inoculation of a human volunteer with a filtrate of infected sheep tissue produced a disease similar to that observed in the herders Epidemiologic evidence pointed to a blood-sucking arthropod, possibly a mosquito, as a vector In 1951 a great epizootic of the disease occurred in South Africa during which it is estimated that 100,000 sheep and cattle died from the disease During this epizootic numerous human beings became infected In 1953 the disease again appeared in epizootic form, and the role of mosquitoes as vectors was clearly established

Clinical Picture

The incubation period is 5 or 6 days Onset is usually abrupt with malaise, chilly sensation and headache The symptoms increase rapidly, and the temperature rises to 102° to 104° F and is associated at times with chills Pain in the extremities and the joints may be extreme There is usually a feeling of discomfort in the epigastrium; definite tenderness or even abdominal pain may be present Nausea and vomiting are sometimes present The face is flushed, and the conjunctivae injected, photophobia is common The temperature curve is, as a rule, of the saddleback type, thus resembling that of dengue and yellow fever The disease usually lasts only a few days Convalescence is rapid, and recovery complete A fatal outcome is rare One of the most characteristic features is a marked leukopenia, which is due chiefly to a diminution of the polymorphonuclear leukocytes The urine, as a rule, is normal Complications are rare, the most important are ocular According to Schrire (1951), central serous retinopathy occurs, occasionally characterized by macular swelling and accompanied at times by small hemorrhages The onset varies from a few days to a few weeks after the pyrexial attack and is associated with a depression of visual acuity With the subsidence of the edema the eyesight generally returns to normal However, in some cases there has been a permanent impairment of vision Of the numerous laboratory infections that have occurred, only one was fatal, due to the development of venous thrombosis 45 days after the onset of the disease (Schwentker and Rivers, 1934)

Pathologic Picture

Human material not being available for study, knowledge of the pathologic picture of Rift Valley fever has been obtained by investigation of material from lower animals In sheep and other animals the most marked changes are observed in the liver (Daubney et al, 1931), focal necrosis, evenly distributed throughout the organ, and pinpoint hemorrhages are present In acute fulminating infections in lambs, the necrosis may be very extensive, destroying almost all the parenchymatous cells The architecture is destroyed, and there is an accumulation of phagocytic cells, chiefly polymorphonuclear leukocytes. The necrosis begins as a hyaline degeneration in a portion of the cell, rapidly extending to involve the whole cell, this process usually commences in the midzone of a lobule At first the degenerating cells are isolated and resemble a similar type of cell seen in livers infected with yellow fever virus and known as Councilman bodies Characteristic of the infection is the presence of intranuclear inclusion bodies Chromatin of the nucleus becomes marginated, and acidophilic material appears in the nucleoplasm. These inclusions are more homogeneous than those seen in yellow fever The pathologic changes observed in other organs are not distinctive. There is a tubular nephrosis; in the spleen and the lymph nodes toxic degeneration is present, and petechial hemorrhages are common in all the viscera Hemorrhagic enteritis has been described

Experimental Infection; Host Range

Sheep, goats and cattle are readily infected with the virus (Findlay, 1932) All monkeys tested to date can be infected, and a nonfatal illness is produced in rhesus, a febrile reaction in South American, and a silent viremia in African monkeys The virus is highly pathogenic for hamsters and is also pathogenic but not invariably lethal for ferrets, rats and other European and African rodents Suckling rats are more susceptible than are adults A species of African field rat (*Arvicanthis abyssinicus*) is readily infected with small inocula of virus and develops a moderately sustained viremia; most of these animals remain well, although a few succumb to the infection (Weinbren and Mason, 1957). Mice are particularly susceptible to the virus, death usually occurring within 2 to 3 days after inoculation In infected mice, the virus occurs in very high titer in the blood, being firmly bound to the red cells (Mims and Mason, 1956). Susceptible animals can be infected by any route, the resultant disease consisting of acute liver destruction Rabbits may develop a viremia of brief duration, while the

horse, pig, guinea pig, chicken, canary and pigeon are resistant

Mackensie and Findlay (1936) and Mackensie et al. (1936) produced a neurotropic strain by inoculating mice intracerebrally immediately after an intraperitoneal injection of immune serum By serial passage in such passively immunized animals, the virus lost to a considerable extent its viscerotropic affinities, so that it no longer produced death due to liver lesions when inoculated subcutaneously Inoculated intracerebrally, this modified virus produced a fatal meningoencephalitis in mice and monkeys A neuroadapted virus also produces a fatal disease on intracerebral inoculation into rats (Findlay and Howard, 1952) Smithburn (1949), by serial intracerebral passage in mice, produced a modified strain of Rift Valley fever virus with marked reduction in its hepatotropic affinites The subcutaneous inoculation of this neurotropic Rift Valley fever virus into newborn lambs or older sheep resulted in the development of immunity, although the virus was not demonstrable in the circulating blood nor did it cause any objective signs of infection Several vaccines have been developed for use in domestic animals (Henning, 1950) The accidental infection of a worker with virus which had undergone at least 300 passages in mice indicates that attenuation for man does not necessarily result from prolonged passage in these animals (Sabin and Blumberg, 1947)

The virus has been shown to multiply in tissue culture of the suspended cell type Both viscerotropic and neurotropic strains are markedly cytopathogenic in tissue culture for sarcoma cells of rat and mouse origin and also for fibroblasts of human embryonic, rat, mouse and swine origin (Takemori et al, 1954)

Etiology

The virus is readily filterable Its size as determined by filtration through gradocol membranes is 23 to 35 mμ, by ultracentrifugation, however, the particle size was found to be 50 mμ (Naude et al, 1954) It can be readily preserved in the lyophilized state

Complement-fixing and hemagglutinating antigens can be prepared readily In the latter, acetone-ether extraction of infective mouse serum has yielded excellent antigens (Mims and Mason, 1956) The virus of Rift Valley fever has no immunologic relationship with any other arbor virus, consequently, the serologic reactions following infection are remarkably specific

Diagnosis

Presumptive diagnosis of Rift Valley fever is made in individuals suffering from an influenzalike fever following contact with the virus in the laboratory or with naturally infected animals A positive diagnosis is made by isolation of virus from blood, this is achieved most readily by the inoculation of mice The virus isolated in this way is identified by typical lesions produced in the liver and by being neutralized by a Rift Valley fever immune serum Virus is present in the blood of human beings during the first 3 days of the disease In convalescence, the diagnosis can be made by demonstrating the development of specific neutralizing or complement-fixing antibodies Neutralizing antibodies have been demonstrated as early as 4 days after onset and as long as 12 years after recovery (Sabin and Blumberg, 1947) It is possible that the hemagglutination-inhibition test may prove to be a useful tool for the diagnosis of Rift Valley fever virus infections

A disease which epidemiologically and in its clinical manifestations in sheep, cattle and humans closely simulates Rift Valley fever is the infection caused by Wesselsbron virus (Weiss et al, 1956) The latter, however, is immunologically a member of Group B and completely unrelated to Rift Valley fever virus

Treatment

This is entirely symptomatic, as there is no specific treatment

Epidemiology

The naturally acquired disease has been [illegible]

Africa The disease is not contagious In-

fected animals can be kept in contact with normal animals without cross-infection taking place Daubney et al (1931) have shown that, in an infected region, sheep can be protected by screening at night From this, it may be concluded that the disease is transmitted by a blood-sucking insect active at night The cessation of an epidemic in a herd removed to the highlands is likewise evidence in favor of insect transmission Smithburn et al (1948), during their studies of the jungle vector of yellow fever in Uganda, isolated Rift Valley fever virus from 6 different lots of mosquitoes caught in the uninhabited Semliki Forest The mosquitoes involved included several species of the genus *Eretmapodites* and 3 of the genus *Aedes* By transmission experiments (Smithburn et al, 1949), it was shown that *Eretmapodites chrysogaster* can act as a vector of the virus. Gear et al (1955) in the studies of an epizootic in South Africa have found *Aëdes caballus* and *Culex theileri* the 2 most prevalent mosquitoes to harbor the virus Naturally infected *Aëdes caballus* were shown to be capable of transmitting the infection to experimental animals by bite *Aedes circumluteolus* has been found naturally infected in Tongaland (Kokernot et al, 1957a) It is apparent that very little definitive information is available concerning the epidemiology of the disease It is probable that domestic animals are infected by some blood-sucking arthropod Man as a rule seems to become secondarily infected during the course of an epizootic The finding of the virus in wild-caught mosquitoes of the Semliki Forest is highly suggestive of some virus cycle in wild animals Antibodies to Rift Valley fever have been found in wild-caught field rats, *Arvicanthis abyssinicus*, in Uganda indicating that these may play some role in the epidemiology and, as previously mentioned, this species is readily infected and develops a viremia How human beings are infected in the laboratory is unknown

Control Measures

Due to the numerous accidental laboratory infections, extreme precautions should be taken by all those working with the virus. There is no specific means of prevention of human infection at present, although vaccines have been developed for veterinary use (Henning, 1956). Arthropod abatement or eradication is perhaps indicated.

CRIMEAN HEMORRHAGIC FEVER

(Synonyms: *Acute infectious capillary toxicosis, hemorrhagic fever of Uzbekistan, of Turkmenia, of Tadjikistan*)

Within relatively recent times, a number of outbreaks of hemorrhagic disease of apparent viral etiology have occurred in various parts of the Soviet Union Various names were given to the outbreaks according to their geographic location, and more than 10 such names have been used in all. In the light of present knowledge, however, the situation appears to be less confusing, and Chumakov has proposed a classification which other Soviet scientists appear to accept (Zeitlenok et al., 1957). According to this concept, there are 3 clearly defined entities: hemorrhagic nephroso-nephritis (the epidemic hemorrhagic fever seen in U. S. military personnel in Korea), Omsk hemorrhagic fever and Crimean hemorrhagic fever The first 2 are dealt with elsewhere in the present volume (Chaps 14 and 20), and only Crimean hemorrhagic fever will be considered here The following description is based on a recent account by Prof M P Chumakov (1957) and upon the review of infectious hemorrhagic fevers in the U.S.S.R by Gajdusek (1953). Some of the information contained in the latter account appears to have been revised considerably since its publication. The first basic data on Crimean hemorrhagic fever were obtained in Crimea in 1944-46 by expeditions under the direction of Chumakov. Subsequent studies have been carried out, in particular those in Uzbekistan by Khodukin and his associates in 1948-1952

The clinical picture is that of an acute systemic disease with sudden onset Malaise, headache, generalized pains, weakness and gastro-intestinal disturbances occur early in association with the initial temperature rise. Fever persists for 5 to 12 days, showing, in the majority of cases, a diphasic course with a short afebrile period on the third to the fifth day Beginning on the fourth to the sixth day, hemorrhagic manifestations appear

consisting of pharyngeal enanthem, petechial rash on the trunk, purpuric skin hemorrhages, bleeding gums, epistaxis and, in severe cases, hematemesis and intestinal, uterine, pulmonary or renal bleeding. Mild or abortive cases occur in which bleeding may be absent. The blood picture characteristically shows a leukopenia which may change to a leukocytosis subsequent to severe hemorrhage. Thrombocytopenia and marked decrease in prothrombin are also characteristic. Crimean hemorrhagic fever is a rather severe infection, and convalescence is slow, but relapses or sequelae are not observed. The mortality rate is given as 2 to 8 per cent.

Pathologic examination of fatal cases discloses hemorrhages into the skin, subcutaneous tissue, lungs and other organs. Massive gastro-intestinal hemorrhage may be found. Degenerative changes in the liver and the ganglia of the autonomic nervous system are described.

Most of the experimental studies have been carried out in human psychiatric patients (the infection being used as a form of fever therapy). The causative agent shows little to no significant pathogenicity for a variety of laboratory and domestic animals. Virus is regularly found in the blood stream of febrile patients, and acute phase serum has been used for a complement-fixing antigen. The virus cannot be propagated serially in monkeys, although it causes a febrile reaction in them. It passes through the usual Seitz and Berkefeld filters and can be stored for long periods in the desiccated state. Using human subjects or monkeys in cross-immunity tests, the identity of various strains of the virus isolated from man and ticks has been established and the lack of relationship shown between this virus and those causing phlebotomus fever (pappataci fever) and Omsk hemorrhagic fever. Complement-fixation studies have also shown no relationship between the agents causing the Crimean and the Omsk diseases. There is, at present, no convenient method for making a specific diagnosis of the Crimean disease.

Convalescent serum is reported to be a successful therapeutic measure for seriously ill patients, treatment is otherwise symptomatic.

The disease has been demonstrated to occur in Bulgaria, the Crimea, the Astrakhan region and the middle Asiatic areas of the Soviet Union. It is a seasonal disease (spring-summer) and rural in distribution, affecting individuals working in or visiting the steppes. The incriminated vectors and reservoirs are *Hyalomma* ticks, in particular *H. plumbeum* and *H. anatolicum*, from which the virus has been isolated. The infection is not normally transmitted directly from man to man, but there has been a significant incidence of intrahospital infections in medical personnel as a result of contact with the blood of acutely ill patients.

Control consists of protection from tick-bite and the use of local tick-control measures.

CALIFORNIA ENCEPHALITIS VIRUS

Three strains of a previously undescribed virus were isolated in 1943 and 1944 by Hammon and Reeves from naturally infected mosquitoes in the San Joaquin Valley of California (Hammon and Reeves, 1952, Hammon et al., 1952, Reeves and Hammon, 1952). The strains were isolated from *Aëdes dorsalis* and *Culex tarsalis* mosquitoes, and a closely related virus was subsequently isolated from *Aedes trivittatus* mosquitoes in North Dakota by Eklund (Hammon et al., 1952). No isolations have been reported from man or other animals.

Hammon and Reeves (1952) reported 3 cases of encephalitis in California which, on serologic evidence, were possibly due to infection with this virus. One patient was an infant, 1 a child, and 1 a young adult. None of the cases was fatal, but the infant experienced a serious illness with possible residua. Serologic surveys in California have shown rather a high incidence (11%) of antibody in human inhabitants of the hot valley areas of the state. Presumably, most human infections are either inapparent or clinically unrecognized. A limited number of specimens obtained from other western states and from Japan were all devoid of detectable antibodies.

The virus is pathogenic for mice, cotton rats and hamsters by the intracerebral route but not for a variety of other animals tested. It reaches a titer of 10^{-5} to 10^{-6} in the brains of infected mice. Rabbits and ground

squirrels show a viremia following subcutaneous inoculation and become immunized. The virus multiplies in the chick embryo but is lethal for only a small proportion of those infected. The size is 60 to 125 mμ by gradocol membrane filtration. Hammon and Reeves reported a minor reaction, detectable by complement fixation, between St. Louis and Japanese B virus antigens and immune serum prepared against California virus. However, Casals (1957) was unable to confirm this finding and has shown that this virus is not a member of the A, the B, or the C Group of arthropod-borne viruses.

A rather high incidence of neutralizing antibody has been found in the sera of horses, cows, rabbits and ground squirrels in some parts of California. Four of 10 species of mosquitoes have been infected experimentally and transmission of the virus shown with *Aedes dorsalis* mosquitoes. No recent evidence is available concerning the role of this virus in human disease.

BWAMBA FEVER

During the course of investigations of jungle yellow fever in Bwamba county in Uganda, Smithburn et al. (1941) isolated 9 strains of virus from the blood of forest workers suffering from fevers of unknown origin. The 9 strains of virus were immunologically identical, and in each instance, serologic evidence was obtained of neutralizing antibodies in the convalescent sera of the donors. Isolation of the virus strains was obtained by the intracerebral inoculation of mice.

The disease was characterized by a sudden onset, fever, headache and backache. The symptoms persisted for about 5 to 7 days. There were no sequelae. Fatal cases have not been seen.

The virus of Bwamba fever multiplies in *Aëdes aegypti* mosquitoes infected by inoculation and can be transmitted from such mosquitoes by bite (Whitman, personal communication). It can be readily maintained by intracerebral passage in mice. On inoculation into *Cercopithecus* monkeys, a rather prolonged viremia occurs (Dick, 1953). The virus multiplies in but is not lethal for chick embryos following yolk-sac inoculation (Taylor, 1952) and it multiplies in and is cytopathogenic for various cell lines, including HeLa, in tissue culture (Moore, 1957). The virus size, as determined by gradocol membranes, is approximately 100 mμ (Smithburn and Bugher, 1953). Antibodies to the virus are found in the sera of individuals over a large area of Africa south of the Sahara desert, and Dick (1953) states that it is probably the most common virus disease in East, Central and West Africa. A high immunity rate has also been found in 6 species of African monkeys, including strictly arboreal ones. The common infection of man and arboreal animals implicates a flying insect as a probable vector (Dick, 1953). Limited surveys for immunity of residents in Asia and South America indicate that this infection is confined to the African continent.

An agent, the Pongola virus, immunologically very closely related to Bwamba, has been isolated on several occasions from wild-caught *Aedes circumluteolus* mosquitoes in the Tongaland region of South Africa (Kokernot et al., 1957b). Immunologically, these two agents are not related to any other arbor virus so far studied.

BUNYAMWERA VIRUS

Bunyamwera virus was first isolated and described by Smithburn et al. (1946). The original isolation was from a mixed pool of *Aedes* mosquitoes captured in the tropical rain forest in Bwamba, Uganda. Subsequent isolations of what appear to be the same agent were obtained from *Aedes circumluteolus* in Tongaland, South Africa (Kokernot et al., 1957a) and from the blood of a febrile child in the same area (Smithburn, personal communication).

Very little is known concerning the clinical manifestations in man infected with Bunyamwera virus. Its ability to cause a febrile reaction is indicated by the single human isolation. In addition, Southam and Moore (1951) inoculated 4 patients having inoperable malignant tumors with a late mouse passage strain of the virus. One succumbed to the malignant growth 3 days after inoculation, and 2 showed no signs of infection. The fourth developed a severe but nonfatal encephalitis attributable to the virus.

The virus is readily maintained by intracerebral passage in mice, producing a lethal infection and high brain titers It is pathogenic for suckling mice by either the intracerebral or the intraperitoneal route. The virus is markedly oncolytic for various mouse and rat ascites tumors in vivo (Koprowski, 1956) and grows well with complete cytopathogenicity in 6 different cell lines in tissue culture (Moore, 1957) Smithburn and Bugher (1953) reported the virus to be 70 to 105 mμ in diameter by gradocol membrane filtration but, on the basis of ultracentrifugation studies, Koprowski (1956) considered it to be much smaller (14 to 22 mμ). Clarke and Theiler (1955) were able to prepare a hemagglutinating antigen from suckling mouse serum, and subsequently a more satisfactory antigen has been prepared by acetone extraction of suckling mouse brain homogenized in sucrose solution By HI and CF tests the Bunyamwera virus is not related immunologically to any other arthropod-borne virus However, Weinbren et al (1957) have presented evidence for some immunologic relationship with a new agent, Simbu virus, recently isolated from *Aedes circumluteolus* in Tongaland, South Africa

Antibodies to Bunyamwera virus have been found in sera from residents of Uganda, Tanganyika, Nigeria (Dick, 1953) and Tongaland (Kokernot et al, 1956) in Africa Limited immunity surveys indicate that human infections with this virus occur in other tropical regions, such as the Amazon valley in South America (Causey and Theiler, 1957) and Malaya and Borneo in Asia (Smithburn, 1954)

GROUP C ARTHROPOD-BORNE VIRUSES

In recent years a large number of virus strains have been isolated in the Amazon rain forest near Belem by Dr O R Causey, director of the Belem Virus Laboratory, Brazil. Isolations have been obtained from the blood of human beings suffering from fevers of unknown causation, from the blood of sentinel monkeys and from wild-caught mosquitoes A large number of the strains are immunologically related to each other but not to any other arthropod-borne viruses These viruses have been studied both by Causey and by Casals and Whitman in the Rockefeller Foundation Virus Laboratories in New York. Although obviously related, these agents are not all identical At least 3 distinct immunologic types can be separated These have been named Marituba, Oriboca and Apeú These agents clearly form an immunologic group and consequently have been designated as Group C by Casals (1957) (Chap 12)

Very little is known so far concerning the clinical aspects of infection with Group C agents beyond the fact that the human isolations have been made in association with febrile illnesses

Viruses of the C group multiply in *Aedes aegypti* mosquitoes, and such mosquitoes can be infected by feeding on experimental animals and can transmit the virus by bite (Whitman, personal communication) The viruses of the group are highly pathogenic for suckling mice, both by intracerebral and peripheral routes of inoculation They show a variable pathogenicity for adult mice and are not lethal for chick embryos Although a moderate virus titer is found terminally in the brains of infected suckling mice, a higher titer is found in blood, and at least one member of the group shows a significantly higher infective titer in liver than in brain Hemagglutinating antigens have been prepared for many of the strains by acetone extraction of suckling mouse serum Complement-fixing antigens are best prepared from infected suckling mouse liver The separation of the agents into distinct species has been made largely by H-I test, these results being, in general, in good agreement with those obtained by neutralization tests With one exception, studies carried out so far by CF tests suggest that there is one complement-fixing antigen common to the C group so that different members cannot be distinguished from each other by this technic

Little information is yet available concerning the incidence of human infection with Group C viruses However, limited serologic surveys with the Oriboca agent indicate that immunity is quite prevalent in residents of the Amazon valley. H-I tests with human sera from Africa indicate that Group C virus infections probably occur on that continent also

OTHER UNGROUPED VIRUSES

Four additional viruses which have not been found to be related to other known agents have been isolated, described and named These are Anopheles A, Anopheles B and Wyeomyia isolated from mosquitoes in Colombia by Roca-Garcia (1944) and Turlock virus isolated by Lennette et al (1957) from mosquitoes and birds in California. At the present time there is not sufficient information concerning the role of these viruses in human infections to justify their further description here.

REFERENCES

Casals, J 1957, The arthropod-borne group of animal viruses, Tr New York Acad Sc, Ser 2, 19, 219-235

Causey, O R, and Theiler, M, 1958, Virus antibody survey on sera of residents of the Amazon valley in Brazil, Am J Trop Med & Hyg 7, 36-41

Chumakov, M P, 1957, Etiology, Epidemiology and Prophylaxis of Hemorrhagic fevers In United States—U S S R Medical Exchange Mission, 1956 Microbiology and Epidemiology Public Health Monograph Number 50 Public Health Service Publication Number 536, Washington, D. C U S. Government Printing Office

Clarke, D. H, and Theiler, M, 1955, The hemagglutinins of Semliki Forest and Bunyamwera viruses, their demonstration and use, J Immunol 75, 470-474

Daubney, R, Hudson, J R, and Garnham, P C, 1931, Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep, cattle and man from East Africa J Path & Bact 34, 545-579

Dick, G W A, 1953, Epidemiological notes on some viruses isolated in Uganda, Tr Roy Soc Trop Med & Hyg. 47, 13-43

Findlay, G M, 1932, Rift Valley fever or enzootic hepatitis, Tr Roy Soc Trop Med. & Hyg 25, 229-265

Findlay, G M, and Howard, E M, 1952, The susceptibility of rats to Rift Valley fever in relation to age, Ann Trop Med 46, 33-37

Gajdusek, D C, 1953, Acute Infectious Hemorrhagic Fevers and Mycotoxicoses in the Union of Soviet Socialist Republics Med Sci Pub No 2, Army Medical Service Graduate School, Washington, D C, pp 6-18, 45-60

Gear, J., et al, 1955, Rift Valley fever in South Africa, South African M J. 29, 514-518

Hammon, W M, and Reeves, W C, 1952, California encephalitis virus A newly described agent, California Med 77, 303-309

Hammon, W M, Reeves, W C, and Sather, G., 1952, California encephalitis virus, a newly described agent II. Isolations and attempts to identify and characterize the agent, J Immunol 69, 493-510.

Henning, M W, 1956, Animal Diseases in South Africa, ed 3, South Africa, Central News Agency

Kokernot, R H, Heymann, C G, Muspratt, J., and Wolstenholme, B, 1957a, Studies on arthropod-borne viruses of Tongaland V. Isolation of Bunyamwera and Rift Valley fever viruses from mosquitoes South African J. M. Sc 22, 71-80

Kokernot, R. H, Smithburn, K. C., and Weinbren, M P, 1956, Neutralizing antibodies to arthropod-borne viruses in human beings and animals in the Union of South Africa, J. Immunol 77, 313-323

Kokernot, R. H, Smithburn, K. C, Weinbren, M P., and de Meillon, B, 1957b, Studies on arthropod-borne viruses of Tongaland VI Isolation of Pongola virus from Aëdes (Banksinella) circumluteolus Theo, South African J M Sc 22, 81-92.

Koprowski, H, 1956, Ascites tumors as culture media in quantitative growth studies of viral agents, Ann New York Acad Sc 63, 895-914

Lennette, E H., Ota, M. I, Fujimoto, F. Y., Wiener, A, and Loomis, E C., 1957, Turlock virus a presumably new arthropod-borne virus Isolation and identification Am. J Trop. Med 6, 1024-1035.

Mackenzie, R D, and Findlay, G M., 1936, The production of a neurotropic strain of Rift Valley fever virus, Lancet 1, 140-141

Mackenzie, R D, Findlay, G M, and Stern, R O, 1936, Studies on neurotropic Rift Valley fever virus the susceptibility of rodents, Brit J Exper. Path. 17, 352-361

Mims. C A, and Mason, P. J., 1956, Rift Valley fever virus in mice. V The properties of a haemagglutinin present in infective serum Brit J Exper Path 37, 423-433

Moore, A E, 1957, Oncolytic properties of viruses Texas Rep Biol & Med 15, 588-602

Naude, N du T., Mason, T, and Polson, A, 1954, Different sized infective particles of Rift Valley fever virus, Nature, London, 173, 1051-1052

Reeves, W C, and Hammon, W M, 1952, California encephalitis virus, a newly described agent III. Mosquito infection and transmission J Immunol 69, 511-514.

Roca-Garcia, M., 1944, The isolation of three neurotropic viruses from forest mosquitoes in eastern Colombia, J Infect. Dis 75, 160-169

Sabin, A. B, and Blumberg, R. W, 1947, Human infection with Rift Valley fever virus and immunity twelve years after single attack, Proc. Soc. Exper Biol & Med 64, 385-389.

Schrire, L, 1951, Macular changes in Rift Valley fever. South African M J 25, 926-930

Schwentker, F F, and Rivers, T. M, 1934, Rift

tropic adaptation of the virus and the experimental use of this modified virus as a vaccine, Brit J Exper Path. 30, 1-16

———, 1954, Neutralizing antibodies against arthropod-borne viruses in the sera of long-time resi-

dents of Malaya and Borneo, Am J Hyg 59, 157-163

Smithburn, K. C., and Bugher, J C, 1953, Ultrafiltration of recently isolated neurotropic viruses, J Bact 66, 173-177

Smithburn, [redacted] C, Haddow, A J., and Gillett, J D, 1948, Rift Valley fever Isolation of the virus from wild mosquitoes Brit J Exper Path 29, 107-121

Smithburn, K C., Haddow, A J, and Lumsden, W H R., 1949, Rift Valley fever transmission of the virus by mosquitoes Brit J Exper Path 30, 35-47

Smithburn, K C, Haddow, A J, and Mahaffy, A F, 1946, A neurotropic virus isolated from *Aëdes* mosquitoes caught [redacted] the Semliki forest, Am J Trop Med 26, 189-208

Smithburn, K. C, Mahaffy, A F., and Paul, J H, 1941, Bwamba fever and its causative virus, Am J. Trop Med 21, 75-90

Southam, C M, and Moore, A E, 1951, West Nile, Ilheus and Bunyamwera virus infections in man, Am J Trop Med 31, 724-741

Takemori, N., Nakano, M, Hemmi, M, Ikeda, H, Yanagida, S., and Kitaoka, M, 1954, Destruction of tumour cells by Rift Valley fever virus, Nature, London 174, 698-700

Taylor, [redacted] M, 1952, Studies on certain viruses isolated in the tropics of Africa and South America their growth and behavior [redacted] the embryonated hen egg, J Immunol 68, 473-494

Weinbren, M P, Heymann, C S., Kokernot, R H, and Paterson, H E., 1957, Studies on arthropod-borne viruses [redacted] Tongaland VII Simbu virus, a hitherto unknown agent isolated from *Aëdes (Banksinella) circumluteolus* Theo., South African J M Sc 22, 93-102

Weinbren, M P, and Mason, P J, 1957, Rift Valley fever [redacted] a wild field rat (*Arvicanthis abyssinicus*) a possible natural host South African M J 31, 427-430

Weiss, K E, Haig, D A, and Alexander, R A., 1956, Wesselsbron virus—a virus not previously described, associated with abortion in domestic animals Onderstepoort J Vet Res 27, 183-195

Zeitlenok, N A, Vanag, K A., and Pille, E R., 1957, Cases of illness of the Crimean hemorrhagic fever type observed [redacted] the Astrakhan oblast Problems of Virology 2, 90-96

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20

Hemorrhagic Fever

(SYNONYMS Epidemic hemorrhagic fever, hemorrhagic nephrosonephritis, Far Eastern hemorrhagic fever, Yaroslavl hemorrhagic fever)

INTRODUCTION

Hemorrhagic fever is an acute infectious disease characterized by physiologic and pathologic disturbances of the vascular system These include capillary dilatation with plasma leakage, proteinuria, petechial hemorrhages and hypotensive shock Toward the end of the first week, about 80 per cent of patients begin their recovery. The remainder have a stormy course with shock, renal insufficiency, marked fluid and electrolyte imbalances and sometimes secondary bacterial complications The mortality is about 5 per cent

The etiologic agent which is filterable can be maintained by serial passage in human beings, who develop typical disease, but it has not yet been established in laboratory hosts The agent presumably causes an enzootic disease in wild rodents and is transmitted in nature by an arthropod vector.

HISTORY

The disease was recognized by Japanese and Russian investigators about 2 decades ago in the environs of the Amur River separating Manchuria and Eastern Siberia (Kitano, 1944; Smorodintsev, 1944). During the Korean War it was an important military problem, being responsible for several thousand cases among United Nations troops (TB Med 240, 1953) More recently, patients with this malady have been reported in several parts of European U S S R (Chumakov et al, 1956, Chumakov, 1957), particularly in the Yaroslavl Region of the Volga Basin A number of other diseases resembling that under discussion have been described during the last decade in Europe and Asia Two of these, Omsk hemorrhagic fever and Kyasanur Forest disease, which are caused by tick-borne viruses of the Russian spring-summer encephalitis group, are discussed in Chapter 19. The others are reviewed elsewhere (Gajdusek, 1953)

CLINICAL PICTURE

Following an incubation period of about 2 weeks, the illness begins abruptly with high fever, chills, prostration, headache, backache and anorexia. Physical findings are limited initially to facial flush and injection of the conjunctivae and the palate. Petechiae appear about the 3rd day, followed promptly by the pathognomonic severe proteinuria The febrile phase usually ends about the 5th day, but manifestations of capillary leakage, including a rising hematocrit, increase during the hypotensive phase which persists for several days The capillary abnormalities begin to regress during the oliguric phase which follows and disappear with the onset of diuresis about the 10th day (TB Med 240, 1953; Earle et al, 1954).

The hypotensive and oliguric phases of the disease are particularly hazardous in the severely ill. In the former, shock occurs with hypotension, decreased cardiac output, reduced circulating blood volume and dilated peripheral capillaries which have reduced flow rate, vasomotor activity and responsiveness to norepinephrine. Certain patients, during the period of resorption of extravascular fluid early in the oliguric phase, develop the syndrome of "relative hypervolemia" with hypertension, high cardiac output, high blood volume, normal or low peripheral resistance, and metabolic acidosis. Acute renal failure with its usual manifestations may develop during the oliguric phase (Entwisle and Hale, 1957, Greisman, 1957).

Leukocytosis of 10,000 to 20,000 with many immature granulocytes usually reaches a peak by the end of the first week. Thrombocytopenia with counts below 100,000 occurs in half the patients. Renal clearance tests show slightly increased values early in the febrile phase, followed by a rapid decrease

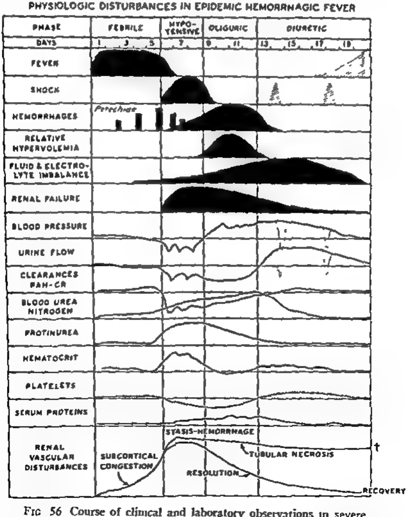

FIG. 56. Course of clinical and laboratory observations in severe epidemic hemorrhagic fever. (Oliver, J., and MacDowell, M., 1957, The renal lesion in epidemic hemorrhagic fever, J. Clin. Invest. *36*, 99.)

during the period of capillary leakage; the values remain depressed in the oliguric phase, then slowly return to normal.

PATHOLOGIC PICTURE

Characteristic lesions are found at autopsy in the pituitary, the right auricle and the kidney Hemorrhages, sometimes massive, occur in the first 2 organs mentioned The pale renal cortex stands out sharply beside the dark-red pyramids. Gelatinous edema occurs in the retroperitoneal tissues and mesentery in patients dying in the hypotensive phase but has disappeared in those who succumb during the late oliguric phase when pulmonary edema is frequently encountered The histologic changes in the kidney are discussed in detail below Other microscopic lesions, except those concerned with hemorrhages visible macroscopically, are meager and consist of scattered small focal areas of necrosis in the viscera. Bacterial pneumonia, when present, presents the ordinary histologic picture. Inflammatory lesions of the vascular tree are conspicuous by their absence, but intense capillary congestion is characteristic (Hullinghorst and Steer, 1953, Lukes, 1954)

The meticulous and beautiful studies of Oliver and MacDowell (1957) provide a correlation of the renal lesions of hemorrhagic fever with the clinical manifestations at different stages of the disease. Figure 56, which presents graphically the principal physiologic disturbances in this malady and indicates the accompanying renal vascular changes, is reproduced from their report

Histologic findings in the kidney in the late febrile and early hypotensive phases are described by these authors as follows. "There is a marked congestive hyperemia localized to the subcortical zone of the medulla, occurring not diffusely but in irregular patchy areas with no intertubular hemorrhage. There are some slight localized pressure effects of this congestion, both of compression and dilatation, on the tubules of nephrons that by chance pass through it, but no structural evidence of general cellular damage in any of the tubules The tubular passages are clear; there are few casts." Later in the hypotensive phase ". . . the evolution of the renal lesion . . . is evidenced by two phenomena; increase in intertubular congestion of the corticomedullary zone, and a swelling of the proximal convolutions The latter has the histologic appearance of an increased hydration of the protoplasm of the epithelial cells which reached the point of vacuolization" As the disease progresses, the vascular disturbances result in increasing damage to the tubules of the nephrons, thus the proximal convolutions in the congested subcortical areas are compressed though their lumens may be filled with hyaline material and desquamated epithelial cells With the beginning of the oliguric phase, true hemorrhages occur in the intensely congested areas. Tubules surrounded by such hemorrhages develop patchy necrosis of the entire wall, including the basement membrane. Furthermore, those portions of such affected tubules which lie in the cortex are dilated with coagulated protein material in their lumens. Such structural changes in a given nephron represent irreversible damage. In patients who recover, one assumes that either the process never reached the irreversible stage or a sufficient number of nephrons escaped anoxic necrosis. Persons dying during the diuretic phase show the usual renal reparative process of epithelial regeneration and replacement fibrosis; moreover, the general dilatation of cortical tubules is not present However, there is no restitution of structure (or function) in those nephrons whose continuity had been destroyed at any point.

Thus, the peculiar renal lesion of hemorrhagic fever is dependent on the same kinds of vascular changes which occur in many other parts of the body, but the response of the kidney is influenced by its unique anatomic structure Capillary dilatation and increased permeability which result from the infection are responsible for the periorbital flush and edema and the cutaneous hemorrhages, and also produce the proteinuria as well as the congestion, edema and hemorrhage in the medullary region With the development of hypotensive shock, hypoxia is intensified in the medullary areas already damaged, and necrosis of tubular portions of nephrons occurs.

EXPERIMENTAL INFECTION; HOST RANGE

Hemorrhagic fever of the type under discussion can be transmitted from man to man by blood, serum, or urine obtained during the first few days of illness Moreover, the infectious agent passes through a *Berkefeld* N filter (Smorodintsev, 1944; Smorodintsev et al., 1953; Kitano, 1944). Attempts by the

Russian and the Japanese investigators just mentioned to produce and maintain a recognizable disease in laboratory hosts with infectious materials were unsuccessful. Repetition of such attempts and extension of the studies to embrace most of the modern technics of virology failed to establish the agent in the laboratory despite the herculean efforts of American investigators of the Commission on Hemorrhagic Fever in Korea and of Russian workers in the Volga Basin (Chumakov, 1957)

ETIOLOGY

Since the transmissible agent of hemorrhagic fever has not been cultivated in laboratory hosts, little is known of the nature of this filterable microbe In studies employing volunteers, Smorodintsev (1944) found that the single convalescent human serum tested was capable of neutralizing the infectious agent No in vitro serologic procedure has been generally accepted as a useful diagnostic tool in this disease

DIAGNOSIS

In the absence of specific tests for the etiologic agent of epidemic hemorrhagic fever, the diagnosis is based on clinical and epidemiologic findings and in the fatal case is confirmed by the characteristic lesions Within the first several days after onset, the illness may be confused with leptospirosis, typhus fevers, idiopathic hemorrhagic purpura, hemorrhagic smallpox, or leukemia Stockard et al (1956) summarized the criteria for diagnosis of hemorrhagic fever as follows "(1) exposure in a known endemic area within 1 to 6 weeks of the onset of symptoms, (2) oral temperature over 100° F persisting for 4 or more days, (3) proteinuria of 0 5 gram per liter on 2 or more consecutive days, (4) hyposthenuria, specific gravity less than 1 024, during the second week of illness by 12-hour Fishberg concentration test or serial 24-hour urine specimens, and (5) one or more of the following petechiae, azotemia with blood urea nitrogen over 25 mg per 100 ml, oliguria with 24-hour urine output of less than 500 ml per day, diuresis with 24-hour urine output of at least 3,000 ml per day for 2 consecutive days, leukocytosis with white blood cell count exceeding 10,000 per μl, or hemoconcentration with hematocrit over 53 ml per 100 ml"

TREATMENT

No specific therapy is available The antibiotics and the drugs effective against bacterial, rickettsial and protozoal diseases are without value in this malady Furthermore, carefully controlled investigations of the Commission on Hemorrhagic Fever failed to reveal therapeutic benefit from convalescent serum Supportive treatment begins with early diagnosis and prompt transfer to a hospital staffed and equipped to cope with a disease characterized by such varied and extensive physiologic disturbances

EPIDEMIOLOGY

Hemorrhagic fever in Korea and the Far East occurs in rural areas throughout the year, but the incidence is greatest during two sharp seasonal peaks, one in the spring and the other in the fall Cases usually appear singly, but small outbreaks do develop, particularly when groups of susceptible persons are exposed in a hyperendemic area Careful study of such outbreaks among UN personnel in Korea has shown that (1) person-to-person infection is lacking, (2) food and water are unimportant in spreading the disease, and (3) all patients in a given outbreak acquire their infection within a day or so while exposed in a small piece of terrain, usually abandoned farmland (Gauld and Craig, 1954)

The American investigators noted the similarity in epidemiologic patterns of hemorrhagic fever and scrub typhus, which is transmitted by trombiculid mites Furthermore, they noted the correlation between the seasonal occurrence of such mites and the incidence of cases of hemorrhagic fever However, because of inability to handle the etiologic agent of hemorrhagic fever in the laboratory, they were unable to prove or disprove their hypothesis regarding trombiculids as vectors in a rodent-to-rodent cycle in nature or in transmission to man (Traub et al, 1954)

The early Japanese workers produced hemorrhagic fever in a volunteer by inoculating a pool of laelaptid mites from wild-caught Manchurian rodents (see Traub et al, 1954).

Chumakov (1957) obtained similar results with Gamasoidae mites collected in rodent burrows near homes of patients in the Yaroslavl region. He favored the hypothesis that human infection occurs from direct contact with excreta of infected rodents The consensus among the different groups of investigators is that wild rodents provide the reservoir of the agent and that mites serving as vectors maintain the infection chains in these hosts There is only indirect evidence regarding the mode of transmission to man

CONTROL MEASURES

In the absence of definitive knowledge about the way in which man contracts hemorrhagic fever, the control measures used by the UN Forces in Korea may be regarded as empirical. Nevertheless, the application of measures against mites and rodents, using the procedures described in Chapter 44, was followed by a sharp decrease in the case rate (Dews and Marshall, 1954)

There is no evidence of communicability of hemorrhagic fever from man to man Hence, isolation of patients and quarantine measures are not required

REFERENCES

Chumakov, M P, 1957, Etiology, epidemiology, and prophylaxis of hemorrhagic fevers Pub Health Monograph No 50, P H S U S Gov't Printing Office, Washington, D C, pp 19-25

Chumakov, M P, et al, 1956, Hemorrhagic fever with renal syndrome in the upper Volga basin, Voprosy Virusologii 4, 26-30

Department of the Army Technical Bulletin TB Med No 240, Epidemic hemorrhagic fever, May, 1953

Dews, S C, and Marshall, I H, 1954, Hemorrhagic fever II. Prevention Am. J Trop Med 3, 601-607.

Earle, D P, (Ed), 1954, Symposium on epidemic hemorrhagic fever, Am J. Med 16, 617-709

Entwisle, G, and Hale E, 1957, Hemodynamic alterations in hemorrhagic fever, Circulation 15, 414-425

Gajdusek, D C., 1953, Acute infectious hemorrhagic fevers and mycotoxicoses in the Union of Soviet Socialist Republics Med Sci Pub No 2, A M S G S, U S Gov't Printing Office, Washington, D C

Gauld, R L, and Craig, J P, 1954, Epidemiological pattern of localized outbreaks of epidemic hemorrhagic fever, Am J Hyg 59, 32-38

Greisman, S E, 1957, Capillary observation in patients with hemorrhagic fever and other infectious illnesses, J. Clin Invest 36, 1688-1700

Hullinghorst, R L, and Steer, A, 1953, Pathology of epidemic hemorrhagic fever, Ann Int Med 38, 77-101

Kitano, M, 1944, Research on epidemic hemorrhagic fever, Manchoukuo M J 40, 191-209

Lukes, R. J, 1954, The pathology of thirty-nine fatal cases of epidemic hemorrhagic fever, Am J Med 16, 639-650.

Oliver, J, and MacDowell, M, 1957, The renal lesion in epidemic hemorrhagic fever, J. Clin Invest 36, 99-222

Smorodintsev, A A (Ed), 1944, Etiologiia i klinika gemorragicheskogo nefrozo-nefrita (Etiology and clinics of hemorrhagic nephroso-nephritis) Moscow, Medgiz 47 pp

Smorodintsev, A. A, Chudakov, B G, and Churilov, A V., 1953, Hemorrhagic nephroso-nephritis, Moscow, Medgiz 126 pp

Stockard, J L, Hale, E H, and Bullard, H V, 1956, Diphenhydramine therapy of epidemic hemorrhagic fever, U S Armed Forces M. J 7, 1405-1413.

Traub, R., Hertig, M, Lawrence, W H, and Harriss, T T, 1954, Potential vectors and reservoirs of hemorrhagic fever in Korea, Am J Hyg 59, 291-305

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21

Rabies

(SYNONYMS Hydrophobia, *rage*, *Tollwut*, *Lyssa*, *rabbia*, *rabia*, *raiva*)

INTRODUCTION

Rabies is a type of encephalitis caused by a virus which exists in nature as a salivary gland infection of carnivorous animals The virus is transmitted from animal to animal and to man by biting All warm-blooded animals are susceptible The incubation period is from 1 to 3 months but may vary from 10 days to 8 months The mortality rate in man is nearly 100 per cent with canine rabies virus

The natural history indicates that the permanent hosts are found in the families *Mustelidae* and *Viverridae* Epidemics of rabies in domestic dogs, foxes, coyotes, jackals, wolves and skunks are the result of the development of large populations of such animals in regions where rabies is present as an inapparent infection in other animals Rabies in vampire bats and insectivorous bats appears to be a recently developed parasitism by the virus

Canine rabies is an example of aberrant parasitism where the natural capacity of the virus to produce encephalitis becomes the means by which it can adapt to the host, that is, by increasing the tendency of this host to bite In wild canines the disease is self-limited because this highly neurotropic virus eventually kills off the host until the chain of infection is broken

In vampire bats rabies virus may be maintained as an asymptomatic infection of the salivary glands. The virus from this host has a different spectrum of pathogenicity from that of canine rabies virus The capacity of rabies virus to invade the salivary glands, breast tissue and kidney tissue makes it possible for the virus to develop cycles of infection in some mammals not associated with encephalitis

HISTORY

Rabies has been known in Europe and Asia since ancient times The history of the disease has been primarily associated with the canine species Knowing that the dog is an aberrant host for the virus, this is a remarkable example of the persistence of basic genetic characters of a virus over a period of more than 2,000 years. Since antiquity the period of summer reckoned by the heliacal rising of the dog star, Sirius, has been referred to as "dog days" when dogs are supposed to be especially liable to spells of mad-

[illegible]

extremely vicious and aggressive without evident cause and, after a period of maniacal behavior, developed convulsive seizures and

paralysis, which were followed by death. Rabies in dogs and domestic animals was described by Democritus (500 B.C.) and Aristotle (322 B.C.). Celsus (A.D. 100) recognized the relationship of hydrophobia in man to rabies in animals and recommended cauterization of wounds produced by rabid dogs. Galen (A.D. 200) favored surgical resection of the wound area

Prior to the 18th century, rabies was known as a disease of wild canines, and domestic dogs played no significant part in its maintenance and spread. Rabies was known in western Europe as early as 1271, when it was prevalent among wolves in France. The first recorded epidemic among domestic dogs in urban centers occurred in Italy during 1708, by 1728 the disease had appeared in epidemic proportions in most of the major cities of Hungary, Germany and France. Rabies was known in England as early as 1613 but did not occur in epidemic proportions among dogs until 1734 Archives of the State of Virginia contain references to rabies in dogs as early as 1753, and those of North Carolina as early as 1762 Rabies was not recognized in South America until 1803 when it appeared among dogs in Peru, in 1806 an outbreak was observed among hunting dogs imported into La Plata, Argentina

The transmission of rabies from a rabid to a normal dog by inoculation of saliva was reported by Zinke in 1804 and by Gruner and by Salm-Reifferscheidt in 1813 These studies showed that the disease was infectious, and it was assumed that destruction of stray dogs and quarantine of other domestic dogs would eliminate the disease. Sanitary measures including these provisions were adopted in Norway, Sweden and Denmark, and by 1826 these countries were free from dog rabies. Although rabies was eliminated from some urban centers in continental Europe by dog-control regulations, these areas frequently became reinfected after a few years. For additional references concerning the early history of rabies, see the 2nd edition

Galtier, 1879, introduced domesticated rabbits as experimental animals for the diagnosis and the study of rabies, but the modern concept of this disease was developed by Pasteur and his associates. The first publication by this group of investigators relative to the etiology of rabies illustrates a problem which has repeatedly confused the study of virus diseases, i e , the isolation of a pathogen unrelated to the disease but transmissible in series in an experimental host. In this case, they isolated an organism from the saliva of a human being with rabies which produced hemorrhagic septicemia in rabbits. The subsequent discovery that the true infective agent of rabies could be recovered from the brain of an animal that died of the disease and the development of intracerebral inoculation by Roux opened the way for an extensive study of the disease (Pasteur et al, 1881). Since the infective agent obtained from brains of diseased animals could not be identified by microscopic examination and could not be cultivated in media used for the growth of bacteria, it was called virus, from the Latin word for poison The ultramicroscopic nature of the infective agent was postulated by Pasteur, but this was not established until Remlinger, 1903, showed that it would pass through Berkefeld filters impervious to bacteria

Pasteur et al (1884) were the first to modify the pathogenicity of a virus for a natural host by serial intracerebral passage in another species of host. In an attempt to develop a variety of rabies virus which could

an infection characterized by a short incubation period; such modified viruses were called fixed, to distinguish them from the natural, or so-called street, virus Though the virus maintained in rabbits at first appeared to become more virulent for dogs, it soon began to show a loss of pathogenicity, and, after 100 passages, the fixed virus had little capacity to infect dogs when given subcutaneously Pasteur had noted that cultures of the chicken-cholera organism, when stored for several weeks at room temperature, lost their pathogenicity but retained their immunizing capacity In an attempt to reproduce this phenomenon with rabies virus, he exposed the spinal cords of rabbits infected with fixed virus to drying at room temperature; then, by means of a series of 10 daily subcutaneous injections of fixed virus, graded from no infectivity to maximum infectivity as determined by intracerebral tests in rabbits, dogs were made resistant to experimental infection with the natural virus. This was a live virus vaccine. During 1885, a peasant boy, who had been severely bitten by a rabid dog, was taken to Pasteur, and, in view of the serious nature of the exposure and the plea that something be done, the boy was vaccinated in a manner similar to that used for immuni-

zation of dogs, the theory being that, if dogs could be immunized in a 2-week period so that they would resist infection with the natural virus, the long incubation period of rabies in human beings would allow the development of a high grade of immunity before the potential onset of the disease The treatment appeared to be without ill effects, and the boy remained well This became known, and other persons were taken to Pasteur for treatment, and the vaccine treatment for rabies soon was adopted as a routine procedure in medical centers throughout the world

Roux, 1887, introduced the use of glycerol as a preservative for maintaining the viability of virus in infected tissue This was applied to the production of vaccine by Calmette, 1891, whereby desiccated spinal cord tissue of varied infectivity could be kept at treatment centers by storage in glycerol Fermi, 1908, was the first to use chemical treatment of suspensions of fixed virus for the preparation of vaccine He introduced the use of phenol for this purpose, and Semple, 1919, by modification of Fermi's method, showed that phenol-treated vaccine could be rendered completely noninfectious and still retain its immunizing capacity, as determined by clinical trial in man

Negri (1903) described the occurrence of characteristic intracytoplasmic inclusion bodies in the nerve cells of human beings and animals proved to have been infected with rabies by isolation of the virus This discovery made possible a prompt microscopic diagnosis of most cases of animal rabies and has been used as a guide for human treatment Though intracerebral inoculation had been successfully employed for many years in the study of rabies in other species of laboratory animals, it was not adapted to the study of the disease in the white mouse until 1930 (Hoyt and Jungeblut, 1930) This animal has been found particularly useful for diagnostic work because of the short incubation period of the disease and the regular production of Negri bodies in its brain following intracerebral inoculation of street virus (Webster and Dawson, 1935)

The development of a method of vacuum desiccation of rabies virus from the frozen state by Harris and Shackell (1911) was a distinct advance Hodes et al (1940) were the leaders in the development of the ultraviolet light treatment of rabies virus for the preparation of a killed virus vaccine

Umeno and Doi (1921) introduced the use of a modified Fermi vaccine, containing active rabbit brain-fixed rabies virus, for the immunization of dogs Results obtained in the large-scale use of the vaccine in dogs in Japan showed that it was effective. A study by Johnson (1945b) on the duration of immunity in dogs vaccinated by the single injection method with a Semple type killed virus rabies vaccine provided the first definite evidence that dogs could be immunized for at least 1 year by this method The experimental method was new, i e, the challenge virus used was derived from the salivary glands of a dog that had died of rabies street virus, diluted and given by intramuscular inoculation so as to approximate natural exposure

Kligler and Bernkopf (1938) were the first to cultivate rabies virus in the developing chick embryo They showed that infection could be produced by inoculation of the chorio-allantoic-membrane and that the rabbit-fixed rabies virus used multiplied in this membrane as well as in the central nervous system of the embryo An avian brain-fixed variety of rabies virus (Flury strain) was developed by Johnson by intracerebral passage in 1-day-old chicks. The character present in the natural virus, which makes it possible for it to invade and multiply in the salivary glands, was eliminated by intracerebral passage for >100 generations in chicks This also reduced the capacity of the virus to invade the central nervous system of the mammalian host following peripheral inoculation The character which makes it possible for this virus to multiply in and destroy adult mammalian nervous tissue was markedly reduced, although not completely eliminated, by >180 serial yolk sac passages in chick embryos (Koprowski et al, 1954a) A single intramuscular injection of the low egg passage (LEP) Flury virus was found to produce a solid immunity to rabies, when tested in dogs by the method developed by Johnson (Koprowski, 1949).

The studies of rabies in vampire bats in Brazil by Queiroz-Lima (1934) revealed that this mammal can transmit rabies for several months as a symptomless carrier This was the first evidence that a mammal may serve as a host of rabies virus under conditions where the virus does not destroy the host In 1953 rabies virus was isolated from insectivorous bats killed in Florida (Venters et al, 1954), Pennsylvania (Witte, 1954) and Texas (Sullivan et al, 1954). This marked the beginning of the first known epidemic of rabies in insectivorous bats.

CLINICAL PICTURE

The onset of the disease is marked by 2 to 4 days of prodromal symptoms, such as fever, headache, malaise, anorexia, nausea and sore throat The temperature is elevated from 1° to 3° F and shows no marked fluctuation. Respiration tends to be shallow, and speech may be interrupted by sighing inspirations The early symptom of most diagnostic significance is some abnormal sensation about the site of infection This will occur in about 80 per cent of the cases and, when present, favors a diagnosis of rabies. The patient may complain of a dull, constant pain referable to the nervous pathways proximal to the location of the wound, or there may be intermittent stabbing pains radiating distally to the region of inoculation

In general, the early symptoms may be ascribed to the stimulative action of the virus affecting various groups of neurons, predominantly those of the sensory system. Though there is apt to be decreased sensitivity to local pain, such as that caused by the introduction of a needle, the patient may complain bitterly of drafts and bed clothes which produce a general stimulation of the skin There is apt to be sensitivity to other types of stimulation such as bright light and loud noise Objective signs include increased activity of muscle reflexes, muscle tics, and general increase in muscle tone Facial expression is apt to be overactive The pulse is rapid Symptoms referable to stimulation of the sympathetic nervous system include dilatation of the pupils, lacrimation, increased salivation and excessive perspiration

In some cases, the excitation phase is predominant up to the time of death However, depressive or paralytic symptoms may be predominant from the beginning or may supervene at any stage of the disease The onset of the excitement phase is gradual and is marked by increasing nervousness, insomnia, anxiety and apprehension There is a strong desire to be up, wandering aimlessly about. A sense of impending death is frequent Despite great fear and anxiety, a patient rarely bursts into tears

The outstanding clinical symptom of rabies is related to the act of swallowing, when fluid comes in contact with the fauces, it is expelled with considerable violence, and painful, spasmodic contractions of the muscles of deglutition and of the accessory muscles of respiration are produced Subsequently, the sight, the smell or the sound of liquids, by suggesting the act of swallowing, may precipitate spasm of the muscles of the throat The name "hydrophobia," or "fear of water," has been used to designate the disease since ancient times because of the frequent occurrence of this symptom in those developing the disease In some cases, the reflex irritability of the throat muscles is not so intense, and the patient exhibits no fear of water, though there may be difficulty in swallowing and a sense of constriction in the throat when the fluid or the food passes the fauces. In order to avoid the act of swallowing, a patient is apt to allow the saliva to drool from the mouth between attempts at expectoration Due to the difficulty in taking fluids by mouth, a patient is apt to develop progressive dehydration, so that the mouth and the tongue become dry and parched. As the disease progresses. the stimulation of the muscular system becomes more pronounced, and vermiform and fibrillar muscular contraction and general tremor may occur. Choking when attempting to swallow may result in such severe spasm of the respiratory muscles that prolonged apnea develops with cyanosis and gasping attempts at respiration. Convulsive seizures are common and may be so extreme as to produce opisthotonus Maniacal behavior, such as tearing clothes and bedding, is not uncommon, but vicious and murderous activity, such as biting and fighting, is rare, though it may occur. Periods of intense excitement are interspersed with those of relative quiet, at which time a patient is well oriented and answers questions intelligently

In the majority of cases a patient dies in the acute excitement phase of the disease during a convulsion Therefore, the paralytic phase caused by degeneration of motor neurons usually is not very evident. However, weakness of muscle groups related to the site of infection may be present early in the course of the disease Ocular palsies, leading to strabismus and in-co-ordination of ocular muscles, may occur. Weakness of the facial and the masseter muscles may be present, so that a patient has difficulty in closing the

eyes or the mouth, and the face becomes less expressive. Weakness of the muscles of phonation may be recognized by the development of hoarseness or loss of voice Examination of the eyes may show partial blindness of the central type, and, though the pupils may have been dilated and may have reacted poorly to light early in the disease, they at times become constricted or show inequality Hippus, nystagmus, diplopia, or strabismus may be noted Vertigo may be present, indicating middle ear disease, and, though this is apt to be an early symptom, it may develop at any period of the disease The corneal reflex is decreased or absent, and the corneae become dry The pulse rate may continue to be rapid with a rate of 100 to 120 at bed rest, but this may shift to bradycardia with a rate of 40 to 60 per minute. Cheyne-Stokes respiration is observed in most cases Though there may be stiffness of the neck, Kernig's and Brudzinski's signs are not elicited A positive Babinski reaction may be obtained Weakness of an extremity is preceded by loss of tendon reflexes Paralysis, when it develops, is of the flaccid type. Local sensation to pin prick, heat and cold is diminished, and in-co-ordination may be noted

If the acute excitement phase is survived, muscle spasms cease, and the patient becomes quiet The fear of water may disappear, if previously present, and the patient may be able to swallow, though with difficulty The face becomes expressionless, and anxiety and excitement are replaced progressively by apathy, stupor and coma In some cases there may be a period of a few hours during which it may seem that a patient is getting better, but this apparent remission is followed rapidly by progressive paralysis Depressive or paralytic symptoms may become predominant at any time during the course of the disease, and in some instances evidence of excitation of the nervous system may be absent Though a patient may complain of fever, headache, nausea and general discomfort at the beginning, the first significant sign is a sudden onset of weakness of one or more extremeties In rare instances, the course of the paralysis follows the pattern of Landry's syndrome and, beginning with the muscles of the legs, a progressive ascending paralysis develops with no relation to the site of the infection Patients developing the predominantly paralytic form of rabies seldom have difficulty in swallowing until the terminal phase of the disease The innervation of the musculature of the bladder and the intestinal tract is affected so that retention and obstipation develop early. Incontinence may develop, especially in patients with a prolonged illness There may be abnormal stimulation of the sex organs. Consciousness is retained until late in the course of the disease Rabies in man, as observed in Trinidad, British West Indies, following vampire bat bite, was uniformly of the paralytic type Nerve root pain preceded or accompanied the spreading paralysis in some cases

The white blood cell count is increased and may reach 20,000 or 30,000 cells per cu mm. Blood smears are apt to show a relative increase in the percentage of polymorphonuclear and large mononuclear cells Examination of the urine may show a slight albuminuria, and hyaline casts may be found in the sediment. A reaction for glucose and acetone is noted in most cases. There is no marked increase in the spinal fluid pressure, but the level ordinarily will be above the average figure. The fluid is consistently clear, though tests may show a slight increase in the amount of protein The cell count usually is within normal limits, more than 100 cells are encountered rarely, if there is an increase, the cells are predominantly of the mononuclear type

If a patient is known to have been bitten by a rabid animal and the symptomatology is characteristic, there is little difficulty in making a correct clinical diagnosis However, in some cases it is impossible to obtain a history of exposure due largely to the failure of a patient or his relatives to recollect a minor wound produced by an apparently healthy dog In such cases, unless the clinical course is typical, the diagnosis may be missed

At times the clinical course of rabies may be very similar to that of poliomyelitis Other viral and rickettsial agents that produce encephalitis or encephalomyelitis must be considered in the differential diagnosis Tetanus may develop following bites by animals, but the incubation period is shorter than that of

rabies, ordinarily from 6 to 14 days. Trismus, though a very constant symptom of tetanus, is rarely present in rabies, and the muscular spasticity in tetanus is constant and general, while in rabies it is intermittent and affects chiefly the muscles of the throat. Where there has been a definite history of dog-bite or other exposure to rabies, it is not uncommon to encounter rabies hysteria In such cases, a patient ordinarily attempts to emulate convulsive seizures. Patients receiving rabies vaccine treatment may develop paralysis attributable to a sensitization caused by the rabbit brain material in the vaccine This paralysis may simulate paralytic rabies and may produce symptoms referable to cranial nerves, such as difficulty in swallowing, paralysis of the masseter muscles and unilateral or bilateral facial paralysis Encephalitis without paralysis may be caused by the vaccine treatment, and in such cases the disease begins with high fever and headache which may be followed by convulsions and coma

The nonfatal or so-called abortive type of rabies described by Koch is uniformly a paralytic disease. On clinical grounds it is impossible to differentiate treatment paralysis occurring as the result of rabies vaccination from paralytic rabies Failure to demonstrate rabies virus in the brain at necropsy is considered evidence in favor of a diagnosis of paralysis due to the vaccine In dealing with animals, however, isolation of the virus becomes increasingly difficult the longer an animal lives after the onset of the disease, and when death is delayed for a week or more it may be impossible to isolate the virus Histologic examination of the brain and the spinal cord in fatal cases of paralysis caused by vaccination will not aid in differentiating the condition from paralytic rabies Furthermore, the problem of differentiating the two conditions cannot be solved by immunologic tests, since the vaccine treatment as well as the disease results in the development of specific antibodies to rabies virus. The concept that rabies in man is always fatal is based on the knowledge that rabies virus has not been isolated from a human being that developed encephalitis and recovered However, the laboratory diagnosis of rabies depends on the isolation of the virus from the saliva, and the testing of saliva for rabies virus has been done so rarely that there are only a few records of isolation of the virus from this source. Of course, a negative saliva test does not rule out rabies infection, since the virus may fail to become established in the salivary glands It is essential to inoculate rabies virus into the brain of the common laboratory animals in order to be certain of producing a characteristic fatal infection. Before the discovery of modern antibiotics, such as penicillin and streptomycin, saliva specimens when introduced into the brain of such animals invariably produced bacterial sepsis Inoculation of the animals by the peripheral route was the only means of testing for the virus in saliva, and the low infection rate and the long incubation period of the disease in animals inoculated by this route made this a tedious and uncertain method of diagnosis It is possible to test saliva specimens for rabies virus by intracerebral inoculation into mice if this material is diluted in physiologic salt solution containing penicillin and streptomycin. This saliva test should be done on all patients that become ill after animal bite and in cases of encephalitis or encephalomyelitis of unknown etiology.

PATHOLOGIC PICTURE

There is slight to moderate ... as shown by flattening of the cerebral convolutions and partial obliteration of the sulci On section, the cut surface of the brain and the spinal cord has a pink cast. Ordinarily, this is more marked in the thalamus, the medulla and the cervical spinal cord When the site of the bite is located on one of the extremities, the cut surface of the spinal cord may show unilateral, pinkish-gray discoloration and obliteration of the normal markings. This lesion, when present, is most marked in the posterior horn. The mucosa of the trachea and the bronchi is congested. The cervical lymph nodes may be slightly enlarged. The thymus may be enlarged and edematous The mucous membrane of the gastro-intestinal ...

eninges

ble de-

gree of hyperemia and slight perivascular infiltration with mononuclear cells may be seen. The cerebral and the cerebellar cortices show general hyperemia and acute neuronal degeneration The white matter exhibits variable demyelinization and degeneration of axis cylinders In the midbrain, the basal ganglia and the pons, the neuronal degeneration generally is severe and is associated with marked hyperemia Small perivascular hemorrhages, most noticeable in the thalamus and subependymal neuroglial tissue, are seen frequently Neuronal degeneration is especially

tion The medulla uniformly presents the maximum pathologic alteration The cranial nerve nuclei exhibit marked neuronal degeneration, and the infiltration by mononuclear cells is proportionally greater than elsewhere The spinal cord shows hyperemia and perivascular infiltration, which are especially marked in the cervical portion at the decussation of the motor tracts The neuronal degeneration is apt to be extensive in the posterior horns When the site of the bite is located on one of the extremeties, the corre-

extensive degeneration of axons and myelin sheaths The contiguity of the axon is disrupted so that it appears beaded, and the myelin sheath is vacuolated The dorsal root ganglia from the same region show marked neuronal degeneration and moderate to marked mononuclear cell infiltration In general, the leukocytic infiltration is largely perivascular, but clusters of mononuclear cells are found about degenerating neurons, especially in the cranial nerve nuclei The leukocytes found in the neuroglial tissue are, for the most part, of the small and the large mononuclear types, but in some cases polymorphonuclear cells are present There is apt to be a slight diffuse mononuclear cell infiltration of the interstitial tissue of the pons, the medulla and the cervical spinal cord, varying in proportion to the degree of neuronophagia Cellular infiltration may be very scanty when a patient dies soon after the onset of the disease and is proportionally greater the longer the duration of the disease The neuroglial cells of the substantia gelatinosa about the central canal show a variable degree of proliferation; this is especially evident in the spinal cord The neuroglia about degenerating neurons become more prominent than usual, and, though this may result from an increase in size, it is suggestive of proliferation. The oligodendroglia throughout the brain show swelling, a manifestation of the moderate cerebral edema which is present in all cases Most of the neurons of the central nervous system show some pathologic alteration The main change consists of pyknosis of the nucleus and ballooning of the cytoplasm The Nissl sub-

coagulative necrosis, while others exhibit fragmentation of the cytoplasm and general loss of cellular detail

The inclusion bodies which can be demonstrated in the neurons of the majority of cases of rabies generally are referred to as Negri bodies These structures when present

defined, spherical, oval or elongated eosinophilic bodies, ordinarily 2 to 10 microns in diameter Several inclusion bodies, usually of variable size, may be present in one neuron and are found most often in the cytoplasm between the nucleus and the dendritic prolongations of the cell They also may be found in the first part of the dendrite and in such instances are elongated The characteristic inclusion body contains an inner structure of basophilic granules, which vary from 0 2 to 0 5 microns in diameter and are surrounded by a clear zone in preparations stained by Wolbach's modification of Giemsa's stain. Large inclusion bodies have a central granule and one or more concentric layers of these inner bodies, separated by a finely granular ground substance or matrix

similarity to inclusion bodies found in other diseases Characteristic inclusion bodies of rabies are apt to be more abundant in Ammon's horn of the hippocampus than elsewhere in the nervous system but may be found in large numbers in the pyramidal cell layer of the cerebral cortex, the Purkinje cell layer of the cerebellum and in the large neu-

rabies, ordinarily from 6 to 14 days. Trismus, though a very constant symptom of tetanus, is rarely present in rabies, and the muscular spasticity in tetanus is constant and general, while in rabies it is intermittent and affects chiefly the muscles of the throat. Where there has been a definite history of dog-bite or other exposure to rabies, it is not uncommon to encounter rabies hysteria. In such cases, a patient ordinarily attempts to emulate convulsive seizures. Patients receiving rabies vaccine treatment may develop paralysis attributable to a sensitization caused by the rabbit brain material in the vaccine. This paralysis may simulate paralytic rabies and may produce symptoms referable to cranial nerves, such as difficulty in swallowing, paralysis of the masseter muscles and unilateral or bilateral facial paralysis. Encephalitis without paralysis may be caused by the vaccine treatment, and in such cases the disease begins with high fever and headache which may be followed by convulsions and coma.

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PATHOLOGIC PICTURE

There are no gross abnormalities which can be regarded as diagnostic of rabies. The surface of the brain and the spinal cord usually exhibits a pink or red discoloration caused by marked engorgement of the blood vessels. There is slight to moderate cerebral edema as shown by flattening of the cerebral convolutions and partial obliteration of the sulci. On section, the cut surface of the brain and the spinal cord has a pink cast. Ordinarily, this is more marked in the thalamus, the medulla and the cervical spinal cord. When the site of the bite is located on one of the extremities, the cut surface of the spinal cord may show unilateral, pinkish-gray discoloration and obliteration of the normal markings. This lesion, when present, is most marked in the posterior horn. The mucosa of the trachea and the bronchi is congested. The cervical lymph nodes may be slightly enlarged. The thymus may be enlarged and edematous. The mucous membrane of the gastro-intestinal tract is congested.

When examined histologically the meninges usually appear to be normal. A variable de-

ity rate of 90 to 100 per cent is obtained if the natural virus is present in high titer in the infected tissue When a 10^{-2} dilution of the same virus preparation is inoculated into dogs in the volume and the manner described, the mortality rate is not reduced markedly, but the mean incubation period may be increased to 40 to 50 days The same type of natural virus, when titrated in dogs by the intracerebral method, may show an LD_{50} of $0.5\ cc \times 10^{-3.3}$ In this instance, the incubation period may be as short as 8 days, but, with rare exceptions, the disease will develop from 10 to 20 days after inoculation, however, a latent period of from 2 to 3 months may be observed The mortality rate following intradermal or subcutaneous inoculation is less than 25 per cent

The signs of rabies in dogs infected by peripheral inoculation are similar to those observed in man For practical purposes, rabies in dogs is classified as furious or dumb type, depending upon the signs shown In the former type, the excitation phase is prolonged, while in the latter, the paralytic phase develops early Most infected dogs show some manifestation of both types, that is, a short excitation phase characterized by restlessness, nervousness and viciousness, followed rapidly by depression and paralysis The incidence of the two types of the disease is not constant and depends on the character of the natural virus Sudden death of dogs from rabies, without appreciable signs of illness, is not uncommon Dogs that develop the predominantly excited type of rabies have a mortality rate of nearly 100 per cent, and the disease usually terminates in death within 3 or 5 days In an exceptional case the dog may live for 2 or 3 weeks from the onset of illness The behavior observed during the prodromal phase of the disease in dogs is of two types most infected animals become increasingly apprehensive and nervous, while others seek solitude and become increasingly apathetic In the former type, a dog may appear unusually friendly, which is probably a manifestation of fear This is the most dangerous phase of the disease as the dog is very excitable and will bite at the slightest provocation A rabid dog is more likely to bite a stranger than its master during the early stage of the disease, but with the onset of the acute excitement phase it may show no recognition of its master and exhibits only an insane desire to attack and bite. The symptom of hydrophobia does not occur in canine rabies, but difficulty in swallowing is common Partial or complete paralysis of the muscles of phonation occurs in most rabid dogs as shown by a characteristic change in their bark or growl or by inability to make any sound This may be the reason why rabid animals so often attack without warning The tendency of rabid dogs to eat dirt, straw, bedding and wood is well known If caged, the animal may break off its teeth in attempts to free itself During the prodromal phase, the physical signs include fever, a decrease or loss of the corneal reflex, decreased sensation to painful stimuli, dilation of the pupils, and an increase in muscle tone which gives the animal an alert appearance As the disease progresses, a dog may be unable to close its eyes completely, and the eyes take on a glazed appearance, resulting from dryness of the corneae An animal may show twitching of muscles, a general muscular tremor, in-co-ordination and convulsive seizures If a dog does not die at this stage, progressive paralysis and coma precede death Dogs infected experimentally by inoculation of virus into the masseter muscles ordinarily develop paralysis of these muscles early, which is spastic at first, making it difficult for the animals to open their jaws after biting Dogs infected by inoculation of the virus into the hind leg muscles usually develop spastic paralysis of the hind legs before other muscles are involved, and paralysis of the jaw occurs late in the course of the disease, if at all The initial febrile period may last 1 or 2 days, and then the temperature drops to normal or subnormal levels A second febrile period is uncommon, but some animals develop a high terminal fever

Transmission of rabies among dogs and related canines depends on the ability of the virus to reach and to multiply in the salivary glands The virus may be present also in the lacrimal glands, the pancreas, the kidney and breast tissue The adrenal glands are likely to contain virus, but cellular degeneration is confined to the medulla and is evidently a part of the general involvement of nervous tissue The virus has not been demonstrated with certainty in the blood, the spleen, the liver,

rons of the basal ganglia and the cranial nerve nuclei They may be found also in neurons of the spinal cord, the dorsal root ganglia, the ganglionic cell layer of the retina, and ganglia of the sympathetic nervous system Negri believed that the intracytoplasmic inclusion bodies of rabies represented one stage in the development of a protozoan parasite Subsequent studies of the inclusion bodies of rabies, as well as those found in other viral diseases, indicate that, while they may contain infective units of the virus, they are composed largely of a matrix derived from the cytoplasm of degenerating cells The absence of thymonucleic acid from the Negri body as shown by the Fuelgen stain conforms with the characteristics of other inclusion bodies due to infection with a virus The matrix contains considerable lipid material as shown by selective staining methods

Neurons of ganglia of the sympathetic nervous system and the dorsal root ganglia of the spinal cord show degenerative changes similar to those of the brain Marked ballooning and vacuolation of the cytoplasm may be present. The interstitial tissue of the ganglia may show moderate to extensive infiltration of mononuclear cells The degenerating neurons are surrounded by large cuboidal cells which appear to be derived from cells of the sheath of Schwann The peripheral nerves, particularly those related to the site of the bite, show coalescence of neurofibrillae and fragmentation of axis cylinders, vacuolation of the myelin sheath and a variable degree of mononuclear cell infiltration in the perineural lymphatics

If the salivary glands contain virus, there

be markedly distended with amorphous ma-

ach mucosa may show acute degeneration. Acute degeneration of the acinar epithelium of the pancreas and the epithelial cells of the renal tubules may be found in some cases of rabies Acute degeneration of the medullary cells of the adrenal gland can be correlated with the presence of the virus and,

the

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may show congestion and edema of the mucosa, and the neurons of the sympathetic plexuses may show degenerative changes sim-

Focal degeneration of the striated muscle has been observed in the tongue

In the absence of specific inclusion bodies in the neurons of the central nervous system, a definite diagnosis of rabies cannot be made with certainty on the basis of the pathologic picture, because the lesions produced by rabies virus are similar to those found in other viral encephalitides

EXPERIMENTAL INFECTION, HOST RANGE

The natural variety of rabies virus as obtained from dogs is pathogenic for all mammals. Injection of the virus into the peritoneal cavity, skin, subcutaneous tissue, muscle, or nervous tissue, in that order, is increasingly efficacious in producing encephalitis. With rare exceptions, intracerebral inoculation of the natural virus obtained from dogs will

encephalitis Intranasal inoculation is almost as effective as intramuscular inoculation in producing the disease Young chickens ordinarily develop a fatal paralytic disease following intracerebral inoculation, but recovery from the disease produced in this way is relatively frequent in mature chickens

The domestic dog is the principal source of human infection, and the nature of the canine street virus can be determined best by study of the disease in this host. A normal dog bitten by a rabid animal may develop rabies within 10 days, or it may show no signs of the disease until several months later. The usual incubation period varies from 21 to 60 days Following inoculation with the natural virus obtained from infected dog salivary-gland tissue, the incubation period depends to a large degree on the amount of active virus introduced. For example, dogs inoculated in each masseter muscle with 0.06 cc of the supernate of a 10 per cent suspension of infected salivary-gland tissue ordinarily develop rabies within 15 or 25 days, with a mean latent period of from 19 to 21 days A mortal-

animals, as well as dogs and large domestic animals such as sheep, horses, calves and goats However, it appears to be unable to produce disease in human beings when given by subcutaneous inoculation A 100 per cent mortality can be obtained in guinea pigs and mice by intramuscular inoculation of large doses of Pasteur rabbit brain-fixed virus Rabies virus adapted to mice by intracerebral passage has an LD_{50} of about 0 03 cc $\times 10^{-7}$ when titrated intracerebrally in mice Such strains of rabies virus have the same pathogenicity range for laboratory animals and dogs as does the rabbit brain-fixed virus. The capacity of the natural virus to form Negri bodies ordinarily is lost following 25 serial intracerebral passages in mice. Some varieties of rabies virus obtained from animals infected in nature are more pathogenic for infant mice than for mature mice. The Flury LEP virus (<50 passages in eggs), when inoculated intramuscularly is pathogenic for mice, hamsters and guinea pigs but not for rabbits and dogs When inoculated intracerebrally it is pathogenic for mice, guinea pigs, hamsters, dogs and monkeys but only slightly so for rabbits The Flury HEP virus (>180 passages in eggs) when inoculated intramuscularly is not pathogenic for mice, guinea pigs, hamsters, cattle, dogs and man When inoculated intracerebrally it is pathogenic for infant mice and monkeys, slightly pathogenic for guinea pigs and hamsters but not for mice, rabbits and dogs (Koprowski et al , 1954a)

Rabies virus can be cultivated in the developing chick embryo. Infection is obtained by chorio-allantoic-membrane inoculation (Kligler and Bernkopf, 1938) and by allantoic sac or yolk-sac inoculation of 7-day-old embryos (Koprowski and Cox, 1948a) The infected embryos are smaller than normal when harvested at the time of peak multiplication of the virus on the 9th day after inoculation, but the embryos seldom die until hatching time, i e , 14 days after inoculation Infection of 1-day-old chick embryos by yolk-sac inoculation results in death of the embryos in 4 to 5 days (Yoshino et al , 1956).

Rabies virus may be cultivated in Maitland-type tissue cultures. Both street rabies virus of dog salivary gland origin and rabbit brain-fixed rabies virus may be cultivated in series in hamster kidney tissue culture (Kissling, 1958).

ETIOLOGY

The diameter of rabies virus has been estimated to be 100 to 150 mμ (Galloway and Elford, 1936) and, as is the case with other large viruses, the agent is not readily filterable It will pass through diatomaceous earth and unglazed porcelain filters which hold back common bacteria but is not readily filterable through Seitz EK pads

Resistance of the virus to various physical and chemical agents depends on the source of the infected material and the method of its preparation. In testing the survival of the virus at various temperatures, one must be cognizant of certain factors, such as the original infectivity of tissues as determined by titration, the percentage and the type of tissue which contains the virus, and the diluent used in the preparation of the material to be tested Infected brain or salivary-gland tissue are the best sources of virus The virus, when infected tissues are stored in undiluted neutral glycerol, retains its infectivity for several weeks at room temperature or for several months at refrigerator temperature. The infectivity of tissues exposed to the air at room temperature is lost in 1 or 2 weeks, depending on the prevailing temperature and relative humidity, but at refrigerator temperature the virus may remain active for several weeks and at subfreezing temperatures for 1 or more years In order to study the nature of the virus and its response to physical and chemical agents, it must be liberated from cells by grinding Intracellular enzymes are liberated with the virus which may have an unfavorable effect on its activity, while certain other substances freed from the cells appear to have a protective effect. In dilutions containing less than 0 1 per cent tissue extract, the virus deteriorates rapidly unless normal serum, or bovalbumin fraction V, is added to the diluent Physiologic saline solution is preferable to distilled water for preparing dilutions of infected tissue, as the latter causes precipitation of protein in cell-free supernates, thus making it difficult to maintain a uniform sus-

the lymph nodes or the muscle of animals infected with the natural virus. The virus is demonstrable in the central nervous system of nearly all animals that die of rabies. When the disease is of long duration, autosterilization may take place The medulla and the thalamus usually contain the greatest concentration of virus. The submaxillary salivary glands are the best source of virus from tissue other than the nervous system and may contain more virus per gram of tissue than the brain The appearance of virus in the saliva coincides with the initial febrile response to infection. Dogs infected by intramuscular inoculation of rabies street virus sometimes develop paralytic rabies and recover. There is no record of isolation of rabies virus from the saliva of a naturally infected dog that eventually recovered

In young hamsters, intramuscular inoculation of the natural virus as derived from infected salivary gland tissue reproduces the disease almost as regularly as intracerebral inoculation (Koprowski, 1949) A 100 per cent mortality can be obtained in guinea pigs inoculated intramuscularly with the virus, but this animal is less susceptible than the hamster when the virus is titrated, using this route of inoculation. White mice, white rats and rabbits have a lower mortality rate than guinea pigs when street rabies virus is given by intramuscular inoculation, and development of paralysis followed by recovery is not uncommon in these animals.

The ability of the virus to invade salivary glands varies with different strains and different species of host Tropism of virus for the salivary glands is markedly reduced by a few serial, intracerebral passages in mice The virus as found in dogs appears to be a highly neurotropic variant of the natural virus. The virus found in vampire bats is less neurotropic, as shown by the development of an asymptomatic salivary gland infection in some bats.

In summary, the canine street-virus rabies is characterized by a long and extremely variable incubation period, a prolonged excitement state associated with irritability and viciousness, and a rather constant production of Negri bodies. In a variable but high percentage of cases, the virus is able to reach the salivary glands and be excreted in the saliva The natural virus may be altered by rapid passage in the canine host as shown by the character of the disease produced, i e, by increased speed of multiplication in the brain, more general distribution of virus in this organ, and less tropism for the salivary glands This undoubtedly acts as a check on the spread of rabies, since animals infected with highly neurotropic strains of the virus uniformly develop a paralytic type of disease and do not propagate it by bite

The term "fixed virus" has been applied to strains of the agent that have been propagated by serial intracerebral passage in some experimental animal, usually the rabbit, in which the incubation period has become short and constant. Rabies caused by fixed virus is characterized by an incubation period of 4 to 6 days following intracerebral inoculation, absence of Negri bodies in the brain, wide dissemination of the virus in the central nervous system with consequent high titer, and a rapidly progressing, paralytic disease Such strains have lost to some degree their ability to migrate along nerves and apparently are unable to multiply in the salivary glands

The variety of fixed virus most widely used for human vaccination is the Pasteur strain, which has been maintained by serial passage in rabbits since its isolation in 1882 (Pasteur, 1885) Most of its substrains, which are available have been through 2,000 or more passages in rabbits The several substrains of the Pasteur strain are not identical in their characteristics The incubation period, the antigen coverage and the ability to invade the central nervous system following peripheral inoculation, differ from strain to strain The method of subpassage in some laboratories evidently has led to the loss of certain characters Titration of fixed virus by the intracerebral route of inoculation shows that the incubation period varies inversely in proportion to the amount of virus given, with a range of 4 to 12 days Incubation periods of 3 to 6 weeks may be observed following intracerebral inoculation of mice with mixtures of rabies hyperimmune serum and fixed virus Subpassage of fixed virus strains obtained by this method indicates that the virus has been altered, probably as the result of elimination of certain characters. The Pasteur strain of fixed virus, when given by intracerebral inoculation, is highly pathogenic for all laboratory

Flury virus in chicks was to eliminate the virus type that could multiply in the salivary glands. The virus was routinely passed during the early phase of multiplication, before the onset of signs of disease in the avian host, and this may have been a factor in the selection of the final virus type. There is a danger in assuming that a certain number of chick embryo passages achieves some definite change in a virus, making it safe for use in a vaccine. This is also true for selection on the basis of pathogenicity for mice when inoculated intracerebrally. The natural virus as derived from Mustelidae is apt to have a greater pathogenicity for infant than for adult mice when inoculated intracerebrally. As shown in studies of the Pasteur strain (Wright and Habel, 1948), the antigenicity of the virus may be altered by subpassage in the rabbit. Vaccines produced from some varieties of this strain do not immunize against other varieties of the same virus. The important thing is whether the vaccine produces protection against the natural virus. This was the basis for the selection of the dog salivary gland virus for testing the immunity of dogs following vaccination. The Flury HEP virus produces immunity in mice to street virus under conditions where immunity is not recognized by challenge with brain-fixed strains.

The site of multiplication of non-neurotropic varieties of rabies virus in the mammalian host is not known. It may be that non-neurotropic variants circulate in the blood stream but cannot be demonstrated because they do not produce encephalitis in the mouse when inoculated intracerebrally. It is not certain whether the virus invades the axonal portion of a nerve tract and follows this to the brain. It may reach the brain via the perineural lymphatics. There is pathologic evidence that the virus produces cytopathology in the lymph nodes, the thymus, the salivary glands, the pancreas and the kidneys. Perhaps the virus may be able to multiply in certain types of muscle tissue. The possibility of a viremic phase in the infection with the natural virus has not been ruled out. Viremia does occur in chick embryos infected with Flury LEP virus (Koprowski and Cox, 1948b).

For details of serologic and immunologic tests the reader is referred to Johnson (1956) and Habel (1954). For the neutralization test, it is preferable to use a standard virus against constant amounts of which 10-fold or half-log dilutions of serum are tested. Testing of undiluted serum may result in nonspecific neutralization of the virus. Immune serum may be prepared by immunization of guinea pigs or hamsters. An initial intramuscular injection of the Flury HEP virus will produce immunity to rabies so that an injection of salivary gland street rabies virus may be given later to obtain a wider antigen coverage. The neutralization test is of value for the identification of unknown strains of rabies virus, particularly those modified by serial passage in laboratory animals or those derived from unusual natural sources.

The immunizing potency of rabies vaccine can be estimated by parallel intracerebral titrations of brain-fixed virus in vaccinated and unvaccinated mice (Webster, 1936, 1939). Rabies vaccines marketed in the United States must be tested for immunizing potency (Habel, 1954; Kaplan, 1954). The antigenic value of the killed-virus vaccine is determined by finding the amount of vaccine required to immunize mice against a standard dose of brain-fixed virus given by intracerebral inoculation. The Flury strain live virus vaccine is tested for potency in guinea pigs. The animals are vaccinated with a single intramuscular inoculation of vaccine, and 3 weeks later the vaccinated animals and the control animals are challenged by intramuscular inoculation with street rabies virus of dog salivary gland origin (Koprowski, 1954a). Mice inoculated intracerebrally with the Flury HEP virus will be immune to subsequent intracerebral inoculation of street virus. This is a convenient method for the identification of rabies virus.

DIAGNOSIS

History of exposure, clinical symptoms and signs, and outcome of an illness often play an important part in the diagnosis of rabies in man and lower animals. The development of specific diagnostic methods for determining whether or not animals have rabies was necessitated by the frequency with which human beings are bitten, especially by dogs which in most instances do not have rabies. If a biting dog fails to develop signs of rabies or to

pension of the virus A large proportion of active virus is found in the sediment following centrifugation of macerated tissue, as shown by regrinding the sedimented tissue and titrating the supernate For routine inoculation, it is preferable to use the supernate, as the suspension of tissue may quickly kill experimental animals when injected into the brain By intraperitoneal or intramuscular injection, a tissue suspension is more infectious than the supernate. Physiologic phosphate-saline, at pH 7 4 to 9, containing at least 2 per cent inactivated normal guinea pig or hamster serum, or 0 75 per cent bovalbumin fraction V, is a satisfactory diluent for studies of rabies virus, there is no significant loss of infectivity in the higher dilutions over a period of a few hours at room temperature or 24 hours at 4° C When exposed to a temperature of from 54° to 56° C, aqueous suspensions of virus are inactivated in an hour or less The best method of preserving the virus is through desiccation while in the frozen state followed by storage at 4° C, under such conditions it may remain infective for many years Dried virus is less sensitive to heat and may prove to be infective after 24 hours' exposure to a temperature of from 54° to 56° C. Repeated freezing and thawing of virus suspensions results in loss of infectivity Variation in subfreezing temperature has a harmful effect on the virus If a standard virus is to be used, it should be stored in pyrex glass ampules, sealed, frozen rapidly and kept in a dry-ice chest Dessicated specimens of rabies virus should not be sealed in vacuum, because there is danger of release of the virus when the ampule is opened Nitrogen gas should be introduced to atmospheric pressure before the ampules are sealed

Rabies virus, as obtained in serum-saline suspensions of infected tissue, may be concentrated and purified to some degree by the common methods of selective precipitation of serum proteins, namely, fractional precipitation with ammonium sulfate and iso-electric precipitation, it is found in the globulin-type fraction Cox et al (1947) reported purification and concentration of rabies virus by means of alcohol precipitation

Rabies virus is rapidly destroyed by sunlight or by ultraviolet irradiation It is readily inactivated by formalin, bichloride of mercury, and strong acids and bases. The virus is moderately resistant to ether and chloroform and very resistant to phenol Bacteriostatic concentrations of merthiolate (thiomersal), sulfadiazine and all the common antibiotics do not harm the virus.

Rabies virus as obtained from infected tissue does not consist of a single type of virus but is a mixture of different types of virus particles, some of which are and others of which are not pathogenic for hamsters and guinea pigs when inoculated intracerebrally (Koprowski et al., 1954a). This phenomenon is observed with several of the Group A arthropod-borne viruses. The neurotropic virus type grows out to become the major population in the first brain passage, but the capacity of the virus to multiply in some types of non-nervous tissue is not lost The Flury HEP virus appears to have a high population of virus particles derived from non-nervous tissue Perhaps, the non-neurotropic virus particles multiply more rapidly than the neurotropic when introduced into the tissues of some hosts and either interfere with infection by the latter or produce immunity to the virus before the neurotropic variety completes its incubation period Passage of rabies virus in chick embryos does not readily change its pathogenicity for the mammalian host For example, Bernkopf and Kligler (1940) noted that rabbit brain-fixed virus after 47 passages in chick embryos was still pathogenic for rabbits when inoculated intracerebrally The Flury virus was still pathogenic for rabbits when inoculated intracerebrally after 50 passages in chicks. It is not necessarily the number of passages in the avian host that brings about the change in the pathogenicity of a virus for mammals. The time of harvesting during the growth period of the virus, the physical conditions of the diluent or variation in the dosage of the virus in the inoculum may be the selective factor. For instance, using a large inoculum, western equine virus remains relatively unchanged in neurotropic qualities following prolonged yolk-sac passage in chick embryos, but the virus derived from the terminal infectivity dilutions of embryo tissue, when used as passage inoculum, is the more neurotropic type This can also be accomplished by a single intracerebral passage in mice The first objective of passage of the

bodies are more abundant in infant mice than in mature mice inoculated intracerebrally with some strains of street virus

TREATMENT

There is no specific treatment for rabies once the disease develops Barbiturates are better than morphine for relieving anxiety, because patients exhibit a marked tolerance for morphine, and small doses actually increase the excitement in some cases If sedatives cannot be given by mouth, phenobarbital sodium given by subcutaneous injection is indicated Anesthesia may be used to control convulsive seizures Dehydration develops rapidly in most patients, this may be controlled by intravenous injection of physiologic salt solution.

Human beings exposed to rabies ordinarily know when the exposure occurred and where the virus was deposited. Local treatment of wounds inflicted by rabid animals has been used for centuries, and this is probably the most important means for preventing the disease The object of local treatment is to remove or inactivate virus that may have been deposited in the wound, and thorough washing with a soap or a detergent solution undoubtedly is the best method of accomplishing this The virus cannot be eliminated from tooth puncture wounds by this cleansing treatment Nitric acid cautery is recommended for treatment of puncture wounds

Vaccination with rabies vaccine, following exposure to rabies by animal bite, is recommended by public health authorities throughout the world. When the incubation period is less than 30 days, which can be expected in about 20 per cent of the cases of untreated rabies, the development of immunity from vaccination may not be rapid enough to prevent the onset of the disease Passive immunization by administration of rabies hyperimmune serum is designed to prevent the development of rabies before immunity is obtained from the vaccine treatment Though partial immunity is obtained in the third week after the beginning of the vaccine treatment, it takes at least 30 days to achieve maximum immunity The studies of Hoyt and Gurley, (1938), Habel (1945), and Koprowski et al. (1950) show that rabies hyperimmune serum given 24 hours after exposure is effective in preventing rabies The effectiveness is demonstrated best in animals exposed to street virus Some protection can be obtained if the serum is given as late as 72 hours after exposure but not later (Koprowski et al , 1950) Clinical studies of the combined serum plus vaccine procedure have not shown 100 per cent protection (Shortt et al , 1935, Proca and Bobes, 1940) The most recent report concerning the use of the serum plus vaccine is that of Baltazard and Bahmanyar, (1955) In August, 1954, a rabid wolf invaded an Iranian village and bit 29 persons before it was killed Eighteen persons were bitten on the head, in the past this type of exposure has resulted in a mortality from rabies of about 40 per cent despite vaccine administration. Five of these received only the Semple type vaccine; the other 13 were given one or more injections of rabies hyperimmune serum beginning 32 hours after exposure, followed by the vaccine Those bitten on the body elsewhere than on the head were given only the vaccine There were 4 deaths from rabies 3 of the 5 bitten on the head and given only the vaccine and 1 given the serum plus vaccine The rabies case in the serum plus vaccine series had an incubation period of 22 days, which shows that the incubation period is not always prolonged following the use of rabies hyperimmune serum Although there is no experimental proof that rabies hyperimmune serum given more than 3 days following exposure will have any protective effect, it is recommended in all severe bite exposures on the basis that it may increase the incubation period so that immunity from the vaccine will have time to develop The occasional failure of the combined serum plus vaccine procedure warrants consideration of using a final dose of street virus of salivary gland origin in the immunization of horses in the preparation of the hyperimmune serum, in order to obtain more complete antigen coverage Rabies hyperimmune horse serum is available commercially as a lyophilized product of good keeping quality and should be kept on hand in public health centers in all areas where rabies is endemic The recommended dosage for the refined hyperimmune serum is 0 5 cc per Kg of body weight given by intramuscular injection

die within a period of 7 days, a bitten person

mals infected in nature, or in the brains of laboratory animals inoculated with saliva or infected tissue from such sources The finding of Negri bodies is sufficient for diagnosis of rabies, but when they cannot be found it is necessary to resort to animal inoculation.

Negri bodies are readily demonstrated in impression preparations of brain tissue stained by Sellers' method (1927). Though Negri bodies usually are more abundant and characteristic in Ammon's horn than elsewhere in the brain, it is advisable to make preparations also from the cerebral and the cerebellar cortices. The impression method is of particular value because the anatomic orientation of nerve cells is retained, and there is little distortion and rupture of cells Ammon's horn is exposed by cutting through the cortex over the posterior horn of the lateral ventricle A cross section is removed from the middle of the horn where it bulges up from the floor of the ventricle, and the cut surface is touched to a glass slide Two or 3 impressions are made, and then the slide is flooded with the staining solution Sellers' stain is a modification of van Gieson's stain and is composed of a mixture of basic fuchsin and methylene blue Negri bodies are stained cherry red and stand out in sharp relief, the basophilic inner structure is colored deep blue The cytoplasm of nerve cells is stained blue, nuclei and nucleoli are deep blue; the stroma is rose pink, nerve fibers are colored a deeper pink, neural sheaths are not stained; bacteria, if present, are stained deep blue, and erythrocytes are copper color For the demonstration of Negri bodies in paraffin sections, brain tissue should

bach's modification of Giemsa's stain as described by Mallory (1938).

In human specimens, there is little chance of confusing the inclusion bodies of rabies with those which occur in other diseases. In dog brains, however, inclusion bodies caused by distemper virus may be encountered which are similar to those occurring in rabies The distemper inclusion bodies are pale red, are more refractile than those caused by rabies and have no inner structure. They may be irregular in outline and occur more frequently in the thalamus and the lentiform nuclei than in Ammon's horn Intracytoplasmic inclusions may be found in the brains of mice which do not have rabies. These are small, pink to bright red in color, uniformly round and have no inner structure. There are cytoplasmic secretory granules in the pons which resemble the inclusion bodies caused by some viruses

Diagnosis of rabies by isolation of the virus depends on the injection into animals of saliva taken during the disease or brain tissue obtained at necropsy Since the submaxillary salivary glands are most likely to contain virus, specimens of saliva should be taken from under the tongue. The saliva may be diluted in a serum-saline mixture containing 500 units penicillin and 1 mg streptomycin per cc. and tested by intracerebral inoculation into mice. Brain tissue obtained at necropsy is prepared for inoculation by grinding in a mortar and adding physiologic salt solution to make a 10 per cent suspension The supernatant fluid following centrifugation, is used for inoculation of mice. Antibiotics should be added to a concentration of 500 units of penicillin per cc and 1 mg of streptomycin per cc. Rabies virus as obtained from dogs ordinarily has an incubation period of 6 to 8 days in mice, following intracerebral inoculation The virus as obtained from wild animals is different in that the incubation period is apt to be 12 to 21 days, and the mice have a more prolonged illness. Most of the mice infected with the natural virus by intracerebral inoculation develop flaccid paralysis of the legs which progresses to complete prostration. It is necessary to confirm a diagnosis of rabies in mice by finding Negri bodies or by the serum-virus neutralization test, because several viruses produce a disease picture in this host similar to that of rabies, and nonspecific intracytoplasmic inclusion bodies are common in mice inoculated intracerebrally with pathogenic bacteria or viruses.

Because Negri bodies become more numerous with progression of the disease, it is advisable to hold biting dogs in quaratine Negri bodies can be found in approximately 90 per cent of naturally infected dogs. Negri

tis caused by vaccine is characterized by high fever, delirium, convulsions and coma which may terminate in death Nonfatal cases ordinarily recover without sequelae The studies of Stuart and Krikorian (1928), Rivers et al (1933), Morgan (1947), and Kabat et al (1947) show that brain tissue functions as an organ-specific instead of a species-specific antigen Therefore, paralysis caused by rabies vaccination must be considered as a specific sensitization to brain material Reactions of the paralytic type are most apt to occur in persons who have had a previous course of rabies vaccine (Horack, 1939, Sellers, 1947). McKendrick (1940) recorded 55 cases of vaccine paralysis, with 14 deaths, among 488,795 persons given Semple type vaccine

The Flury LEP live-virus vaccine has been shown to be superior to Semple type killed-virus vaccine for the immunization of dogs (Koprowski and Black, 1952, Tierkel et al, 1953) The Flury LEP virus has been used on a large scale in dogs, and there is no evidence of paralytic reactions from sensitization to the nervous tissue in the chick embryo This experience with the Flury LEP virus in animals and the development of the Flury HEP virus which has very little pathogenicity for mammals has prompted investigation of the use of Flury HEP virus for immunization of man Inoculation of mice, guinea pigs, dogs and cattle with Flury HEP virus produces immunity to infection with street virus The protection ratio for calves was the same whether 3 cc or 15 cc of the Flury HEP vaccine virus was given by intramuscular injection, indicating that the 3 cc dose was sufficient to immunize a calf (Koprowski and Black, 1954b) The Flury HEP virus has been tested in more than 1,000 human subjects where the vaccine was given by intramuscular or intradermal injection, and there have been no serious reactions (Fox et al, 1957, Sharpless et al, 1957) The effectiveness of the vaccination procedure used in the tests in man has been determined by testing the blood serum of vaccinated subjects for virus neutralizing substance Studies of immunization of dogs with Flury LEP live virus vaccine and Semple type killed-virus vaccine have shown that it is not uncommon to have immunity to street virus, as determined by challenge inoculation, in dogs whose serum contains no readily demonstrable virus neutralizing substance (Koprowski and Black, 1952; Johnson, 1954). The serologic method of rating a vaccine may result in accrediting a nonprotective vaccine because of the demonstration of nonspecific virus neutralization in the test as done and eliminating a protective vaccine because of the failure to demonstrate regular production of virus neutralizing substance in the blood of the vaccinated subjects. The results obtained in serum-virus neutralization tests with brain-fixed test virus may be different from those obtained with street virus (Johnson, 1948). The important thing is whether a vaccine produces immunity to infection with the natural virus The observation by Kissling (1958) that both brain-fixed and street virus will multiply in non-nervous tissue warrants consideration of the possibility that Flury HEP virus may multiply in man A certain minimum dosage is needed when a vaccine virus is inoculated into the muscle tissue in order to distribute the virus to susceptible tissues away from the point of inoculation as well as to seed the virus locally Vaccination by the intramuscular route with Flury LEP virus has been shown to be very effective in animals; until intradermal injection of the Flury HEP virus has been shown to produce immunity to the natural virus in dogs, monkeys or other large animals, it is not recommended for prophylactic immunization or postexposure vaccine administration in man All variations in methods of production of experimental live-virus or killed-virus vaccines for study in man and lower animals should be checked by submitting each lot of vaccine to protection tests in animals using street virus of salivary gland origin for the challenge virus Variation in dosage of the inoculum, route of inoculation, age of the chick embryo host and portion of the chick embryo used may be expected to yield differences in the ratio of various types of virus particles in the live virus vaccine. The important thing is to have a sufficient amount of the virus type in the vaccine which produces immunity to the neurotropic variant of the natural virus On the basis of animal experimentation it is reasonable to expect a high degree of immunity in man from a single intramuscular dose of 3 cc of Flury HEP vaccine virus of standard titer When used for

in the buttocks Skin testing must be done with the rehydrated serum diluted to 1 1,000 in physiologic salt solution, before giving the entire dose Persons showing skin sensitivity to horse serum may be treated by the desensitization procedure, giving injections of much-diluted serum and increasing the dosage at 20-minute intervals until the entire dosage is given Acute reactions, such as syncope, generalized urticaria, or angioneurotic edema, following the injection of horse serum, ordinarily may be relieved by injection of adrenalin and use of antihistaminics Acute reactions such as this and also the delayed serum sickness are uncommon in children, but the delayed serum sickness reaction is common in adults.

For persons known to have been bitten or scratched, vaccine or serum plus vaccine should be started immediately. (1) when the animal is apprehended and presents clinical signs of rabies, (2) when the animal is killed and the brain is found to be positive for rabies by microscopic examination, (3) when the animal is killed, and, though the brain is negative by microscopic examination, the animal is suspected of being rabid, and (4) when a person is injured by a stray animal that escapes or by one that cannot be identified Vaccine is rarely indicated when there is no satisfactory evidence of a person having been bitten The immediate administration of hyperimmune serum followed by vaccine beginning 24 hours later is recommended for all persons receiving severe bite exposure from rabid animals.

There are 2 types of killed-virus vaccine which are recommended (1) The Semple type packaged in 7 or 14 doses of 2 cc of 5 per cent or 0 5 cc of 20 per cent rabbit brain tissue infected with the Pasteur rabbit brain-fixed virus in physiologic salt solution containing 0 25 per cent phenol The virus is inactivated by incubation at 37° C The recommended procedure consists of 14 daily injections of vaccine given into the subcutaneous tissue of the abdominal wall, a different site should be used for each injection (2) The ultraviolet-light-irradiated or U V vaccine is similar to the Semple vaccine It is packaged in 7 doses of 2 cc of 5 per cent or 1 cc of 10 per cent rabbit brain tissue infected with the Pasteur rabbit brain-fixed virus in physiologic salt solution In this vaccine the virus is inactivated by ultraviolet irradiation. Merthiolate (thiomersal) at a 1:8,000 dilution or phenol at 0 25 per cent is added to the tissue suspension as a preservative. There is little difference in the antigenic qualities of the 2 killed-virus vaccines, and both have to pass the same antigen potency test

Vaccine should not be given unless there is good evidence of exposure to rabies. Sensitization to rabbit-brain tissue may produce serious allergic reactions Acute reactions, such as syncope, generalized urticaria, or angioneurotic edema, may occur soon after an injection of vaccine in persons who have been sensitized by previous vaccination. Persons who have not been sensitized previously are not apt to show any reaction until 7 or 8 days after the first dose The most common reaction is the development of erythema and edema about the site of vaccination with accompanying pruritis and pain These reactions tend to subside in a few days despite the continuation of treatment If local reactions are accompanied by fever, headache, nausea, lymphodenopathy and malaise, it is wise to stop the treatment This type of reaction is uncommon but usually precedes the development of more serious complications, such as encephalitis and paralysis. The paralytic phenomena, which may follow the administration of vaccine, include peripheral neuritis, dorsolumbar myelitis, and paralysis of Landry's type. Reactions characterized by paralysis seldom develop until 5 days after the first dose but may occur as late as 2 weeks after the completion of treatment. The peripheral neuritis most often involves the facial nerves, but other cranial nerves may be affected, recovery usually occurs in 2 or 3 weeks. Individuals who develop the dorsolumbar myelitis ordinarily recover This reaction is characterized by fever and gradual onset of weakness, numbness and tingling of the lower extremities, there may be urinary retention In cases of paralysis of the Landry's type, the onset is abrupt with high fever, headache, nausea, vomiting, girdle pain, urinary retention and ascending paralysis The paralysis may extend to involve the bulbar nuclei and terminate fatally More often the patient recovers rapidly, though in rare instances there may be permanent disability. Acute encephali-

demic of dog rabies in southern Rhodesia in 1902. Subsequently, this region was free of dog rabies until 1950 when the disease appeared among dogs along the border adjoining Bechuanaland This epidemic was controlled by the immunization of domestic dogs with the Flury LEP live-virus vaccine, but the disease is known to be present in wild life, and the existence of rabies in civet-cats, *Civettictis civetta*, has been confirmed by laboratory studies A few polecats, *Ictonyx striatus*, were diagnosed as rabid on the basis of observations (Adamson, 1954)

Rabies is especially common in India, for example, Veeraraghavan (1956) reported 1,475 human cases of hydrophobia in Madras State during the year 1941, and an incidence of more than 1,000 cases each year in this State through 1944. In 1954 there were 102 cases of human rabies in Madras State. The jackal, the fox and the wild dog are the common wild canine vectors of rabies in India The mongooses of the subfamily, *Herpestinae*, and the civets of the subfamily, *Viverrinae*, probably form the more important wild-life reservoir of the disease in India and elsewhere in Asia In Japan, rabies occurred in epidemic proportions in dogs in 1924, and there were 235 human deaths from rabies that year Vaccination of dogs with a Fermi type live-virus vaccine, using the rabbit brain-fixed virus, resulted in a sharp reduction in the incidence of dog rabies, and in 1932 only 63 cases of rabies were identified in dogs, and no human cases were reported (McKendrick, 1933) At the present time rabies does not appear to be established in domestic dogs in Japan Rabies has not been reported in Australia or the Hawaiian Islands It is of interest to note that the animal family of *Mustelidae* is not represented on these islands

In North America rabies was common in dogs in the New England States during the latter part of the 18th century The disease is known to have been epizootic in foxes in Massachusetts during the first decade of the 19th century (Thacher, 1812), in Alabama in 1890 (Wilkinson, 1894) and in Alaska in 1915 (Ferenbaugh, 1916) Since 1940, rabies has again become prevalent in foxes in eastern and southern United States (Johnson, 1945a) The focus of fox rabies which developed in New York State in 1945 (Korns and Zeissig, 1948) is still active, although dog rabies has been controlled effectively since 1947 In 1915 and 1916 rabies appeared in epidemic proportions in coyotes in California, Oregon and Nevada (Mallory, 1915; Geiger, 1916) It is the history of skunk rabies that suggests the true nature of rabies in wildlife in North America The earliest reference to skunk rabies in North America is that of Duhaut-Cilly who, when he visited Lower California in 1826, noted that the people there told him that skunks sometimes entered houses and bit people and gave them hydrophobia (Nelson, 1918) An epidemic of skunk rabies, which began in Kansas in 1873, was responsible for the death of at least 40 persons, mostly cowboys and hunters, who were bitten by rabid skunks when camping on the plains (Seton, 1925). Another epidemic of skunk rabies occurred in Arizona; it began in 1907 and continued through 1910, and at least 10 persons died after they were bitten by skunks (Yount, 1910) The small spotted skunk, *Spilogale putorius*, appears to have been the most frequent source of human infection, these animals are often referred to as phobey cats in western United States, because it is common knowledge that they have been the source of hydrophobia in human beings Although the recent epidemic of rabies in wildlife began in 1940, there was a sharp increase in incidence of the disease in wild animals in 1953, involving a great variety of animal species Skunk rabies appeared in epidemic proportions in Iowa, Minnesota, South Dakota, Texas and Wisconsin in 1953 and in California in 1954 Skunk rabies was reported in 24 states in 1955 (Chadwick, 1954, 1956) During epidemics of skunk rabies the principal vector in the striped skunk species, *Mephitis mephitis*, based on the recognized cases of rabies However, this may be more apparent than real, because this skunk is more closely associated with human habitation, as compared with the small spotted skunk, *Spilogale putorius*. There have been isolated cases of rabies in mink and weasels of the *Mustela* genus

The year 1953 marked the first time that rabies was identified in insectivorous bats in the United States During that year rabies virus was isolated from bats killed when attacking persons in daytime in Florida (Venters et al, 1954) and Pennsylvania (Witte, 1954) and from bats collected in Texas (Sullivan et al, 1954) Subsequently, rabies has been reported in insectivorous bats in 8 states (Enright, 1956) The species most commonly infected are the free-living, migratory, insectivorous bats of the *Dasypterus* and *Lasiurus* genera and the colonial, cave-dwelling Mex-

prophylactic immunization this should be sufficient for primary immunization, and a second or booster injection is recommended after exposure. For those not previously vaccinated there is a special problem in the use of a live virus vaccine, that is, the use of hyperimmune serum may interfere with the production of immunity from the vaccine because of inhibition of virus multiplication On the basis of results obtained in serum-virus neutralization tests of blood serum from vaccinated subjects there is some evidence that the prior use of hyperimmune serum interferes with the production of virus neutralizing substance by either killed-virus or live-virus vaccine (Atanasiu et al, 1956) However, Koprowski (1954b) found that the combination of hyperimmune serum and Flury LEP live-virus vaccine was more effective for post-exposure protection in dogs than either serum or vaccine alone The evidence at hand seems to be sufficient to warrant recommendation of the Flury HEP live-virus vaccine for use in man as a prophylactic vaccine for those subject to high risk of exposure to rabies. Its use for postexposure protection is indicated for those that have had previous administration of brain tissue vaccine and those that develop signs of sensitization to the brain tissue vaccine. Studies of various schedules of inoculation in dogs, monkeys or other large animals are indicated to determine the best dosage and spacing of injections for post-exposure protection with Flury HEP live-virus vaccine used alone and following hyperimmune serum.

EPIDEMIOLOGY

There are two epidemiologic types of rabies: the natural disease as it occurs in the wild animal host and the encephalitic type which is maintained in dogs. The current world-wide distribution of rabies is due to the general popularity of dogs as pets Domestic dogs revert easily to a semiwild or scavenger-existence, and stray dogs increase rapidly in any urban community unless an organized effort is made to destroy them

The early history of rabies in Europe indicates that the disease was endemic in certain regions which served as starting points for recurrent migrating epidemics of wolf and fox rabies The disease was limited by certain geographic barriers, such as mountain ranges and water, as well as by the abundance and the distribution of suitable hosts capable of spreading the infection Once established in domestic dogs, these limiting factors were much less effective, as dogs could travel with their masters from city to city and from country to country, furthermore, these animals were sufficiently numerous in any large city to maintain the disease once it was introduced. Dog rabies became very prevalent in Europe during the first half of the 19th century (Koch, 1930). In recent years the incidence of the disease has been low in western Europe; and the British Isles, The Netherlands, Denmark, Norway, Sweden and Switzerland have remained free of dog rabies

Rabies is known to exist throughout southwest Asia The disease has been known for a long time in Kenya and occurred in epidemic proportions among jackals from 1912 to 1916 (Hudson, 1944). A disease of dogs, known as oulou-fato, which exists in West Africa, has been identified as rabies, but it seems improbable that the disease is maintained in dogs or related wild animals of the family *Canidae*, in this region Human rabies is seen rarely in West Africa, and the disease is uncommon in dogs (Nicolau et al, 1933) Furthermore, the occurrence of paralytic rabies in dogs that are kept confined in compounds and not exposed to other dogs suggests that a small mammal is the vector in West Africa. In 1949, a variety of rabies virus of the oulou-fato type was isolated in the Belgian Congo (Delville and Jezierski, 1950). Rabies was recognized for the first time in South Africa in 1892 when it appeared in dogs in and around Port Elizabeth. Subsequently, there were occasional outbreaks of dog rabies which were brought under control quickly by rigid dog control regulations Between the outbreaks of dog rabies there have been repeated instances of human infection with rabies from the bite of the yellow mongoose, *Cynictus penicillata*, and this animal is considered to be the principal wild life vector in South Africa (Snyman, 1937) However, it may be that other members of the subfamily, *Herpestinae*, or the subfamily, *Viverrinae*, are the natural hosts of the disease. On the basis of general ecology, the cape polecat, *Ictonyx striatus*, the African weasel or snake mongoose, *Poecilogale albinucha*, and the civet-cat, *Civettictis civetta*, would seem to be the more likely hosts of rabies in South Africa There was an epi-

eral involvement of wildlife with rabies An abundance of susceptible hosts is a necessary feature of the development of epidemic rabies The increase in population of vampire bats in Mexico, Central and South America was brought about by the development of large cattle ranches, which furnished a ready source of blood for this hematophagous mammal The type of infection which developed in the vampire bat is a blind end in the ecology of the virus in that it is not perpetuated by the animal infected by bat-bite, i.e., they develop the paralytic form of the disease and do not become vicious and bite Therefore, the virus may have a cycle of infection which is like that of the true natural host where the perpetuation of the virus does not depend on the development of encephalitis in the infected animal, for instance, transmission by a cycle involving the infection in the breast tissue or the kidney One can assume that infection by bite in fighting among bats would not be so apt to produce the nonencephalitic type of infection noted in the vampire bat

The ecology of weasels of the *Mustela* genus is not so very different from that of the vampire bat The weasel exhibits a preference for feeding on blood They are good climbers and would likely prey on bats found in treeholes and on the branches of trees Vampire bats living in hollow trees would be likely prey for weasels, and colonial bats which live in caves would be taken by this animal as well There are many members of the families *Mustelidae* and *Viverridae* which have habits similar to that of the weasel in North America The small spotted skunk, *Spilogale putorius*, evidently plays an important part in the ecology of rabies virus in North America This skunk is a good climber and could thus serve to spread the infection to bats

The existence of rabies virus in the families *Mustelidae* and *Viverridae*, may be the equivalent of a built-in antibiotic against the carnivores of the family *Canidae* The virus does seem to be a major factor in controlling the population of wolves, foxes, jackals, coyotes and wild dogs There is the possibility that the virus is a natural disease of small mammals such as voles and bats, and that they perpetuate the virus during interepidemic periods, but this seems unlikely on the basis of present knowledge of the epidemiology of rabies

There has been a sharp reduction in the incidence of dog rabies in the United States during the past few years The enforcement of proper dog-control regulations and vaccination of domestic dogs with the very effective Flury LEP live-virus vaccine can be expected to eliminate dog rabies from any community within a few months The incidence of rabies in man has been reduced also; for example, in 1908 there were 111 human rabies cases in the United States (Stimson, 1910) compared with only 5 cases of rabies in man recorded in 1955 (Chadwick, 1956)

The attack rate of rabies in human beings bitten by rabid animals depends on several factors, for example, a rabid animal may not have the virus in its salivary glands, protection provided by clothing may be so great that little or no virus enters the wound produced, the natural source of the virus may be such that it is not very infectious for man, and virus may be removed from open wounds by cleansing with soap and water Nevertheless, the susceptibility of man to canine rabies appears to be relatively great Fetherston and Cooper (1932) observed 4 cases of rabies in 8 sailors bitten on their hands by a stray dog which they had picked up in a Chinese port and taken on board a destroyer, the bites of wolves are particularly dangerous as shown by the statistics given by Baltazard (1954), who recorded 60 rabies deaths, a mortality rate of 18 per cent, among 325 persons bitten by rabid wolves and given postexposure vaccine, and 53 rabies deaths, a mortality rate of 28 per cent, among 186 persons bitten on the head, despite vaccine administration Veeraraghavan (1954) arrived at a mortality rate by including only those bitten by an animal proved to have transmitted rabies to persons or animals In this category he recorded 19 rabies cases, a rate of 43 per cent, among 44 persons that did not receive the vaccine, and 10 rabies cases, a rate of 8 per cent, among 120 persons receiving a full course of vaccine The most extensive statistics as to attack rate of rabies in man are those of Schuder which, as quoted by Kraus et al (1926), list 1,325 rabies deaths in a group of 14,959 persons bitten by rabid animals, a rate of 9 per cent This information was obtained from records antedating vaccine prevention Of the first 214 patients with face bites treated at the Pasteur Institute in Paris, 12 or 5 6 per cent died of rabies despite vaccine administration The danger of bites about the head and the face is great, as shown by the statistics reported by Kraus et al (1926), a mortality rate of 11 per cent for children and 3 per

ican freetail or guano bat, *Tadarida mexicana*. The occurrence of epidemic rabies in Mexican freetail bats at Fort Sam Houston, Texas, and Carlsbad Caverns, Carlsbad National Park, N. Mex., in 1954, reported by Burns et al., (1956) is of particular interest because this bat is known to migrate to Mexico in the fall, returning the next spring (Villa, 1956). A *Tadarida mexicana*, banded at Carlsbad Caverns on September 18, 1952, was recovered in the caves of Las Garrochas, near Soyatlan del Oro, Jalisco, Mexico, on November 25, 1952, a flight distance of 800 miles. Another *Tadarida mexicana*, banded at a cave near Monterrey, Nuevo León, Mexico, January 29, 1956, was recovered at Carlsbad Caverns, N. Mex., on August 27, 1956, a flight distance of approximately 730 miles (Villa, 1956).

The history of rabies in the Far North goes back to 1870 when the disease was recognized in Greenland (Stimson, 1910). Subsequently, there were reports from time to time of a disease like rabies in wolves, foxes and sled dogs. Beginning in 1947, rabies was reported in foxes and wolves in widely scattered foci in the Northwest Territories. It was soon recognized that the disease was present throughout the entire Northern Territories of Canada, and the disease was identified as rabies by laboratory studies (Plummer, 1954). Rabies has also been identified in foxes and dogs in Alaska (Chadwick, 1956). Rabies is not recognized in the Arctic until it appears among foxes, wolves and sled dogs, but these animals do not seem to be able to perpetuate the disease in this region. Epidemics of rabies in the Far North coincide with periods of high population of lemmings of the *Lemmus* or *Dicrostonyx* genera and voles of the *Microtus* genus. The increase in population of the smaller mammals, in turn, results in an increase in the population of carnivorous animals which feed on them, and this may be the precipitating factor which produces epidemics of rabies. Of the *Mustelidae* in the Far North, it is the weasel or ermine, *Mustela erminea*, whose ecology suggests that it may be the natural host of rabies in the arctic regions.

Rabies has been identified in the mongoose species, *Herpestes auropunctatus*, in Puerto Rico (Chadwick, 1956), and it is of interest that this animal is not indigenous to this island but was introduced in the latter part of the 19th century as a means for controlling rodents.

The discovery that vampire bats are infected with rabies in some sections of Mexico, Central and South America is of considerable epidemiologic importance in that this animal is known to transmit rabies as a symptomless carrier. True vampire bats are found only in Mexico, Central and South America. The existence of rabies among vampire bats was recognized first in the State of Santa Catharina, Brazil. A paralytic disease of cattle and other livestock called *mal de caderas* appeared in epidemic form in that region in 1908. Some of the animals were proved to be infected with rabies virus (Carini, 1911). From the beginning of the outbreak, ranchers had observed that bats were flying in the daytime and fighting one another—unusual pheonomena—and that cattle bitten by bats during the day ordinarily developed paralysis and died within a few weeks. In 1916, rabies virus was isolated from a bat captured while feeding on cattle during the day (Haupt and Rehaag, 1921). On the basis of this finding, it was concluded that the repeated epidemics of paralytic rabies, which occurred in livestock in Brazil from 1908 to 1918, were due to infection induced by the bites of vampire bats. During the period 1931-1934, there was another outbreak of paralytic rabies in Brazil; rabies virus was isolated from naturally infected vampire bats, *Desmodus rotundus*, and this bat was shown to be able to transmit rabies for a period of 5 months as a symptomless carrier (Queiroz-Lima, 1934). A paralytic disease of cattle and man appeared in Trinidad, B.W.I., in 1925, but the disease was not identified as rabies until 1930 when the virus was isolated from a patient diagnosed as poliomyelitis (Hurst and Pawan, 1931, 1932). Subsequently, it was shown that the vampire bat, *Desmodus rotundus*, was the vector of rabies in Trinidad (Pawan, 1936a). The occurrence of an asymptomatic virus infection of the salivary glands in the vampire bat was confirmed by Pawan (1936b). Fifty-five cases of human paralytic rabies in Trinidad, presumably the result of bat-bite, were recorded by Verteuil and Urich (1936). In the course of a field study in the State of Michoacan, Mexico, during 1944, active agents later identified as rabies virus (Giron, 1944; Johnson, 1948) were isolated from a paralyzed cow and from the salivary glands of vampire bats captured in a cave located near the place where cattle were dying of paralysis.

Rabies in bats seems to be an aberrant cycle of the disease, part of the recent gen-

eral involvement of wildlife with rabies An abundance of susceptible hosts is a necessary feature of the development of epidemic rabies The increase in population of vampire bats in Mexico, Central and South America was brought about by the development of large cattle ranches, which furnished a ready source of blood for this hematophagous mammal The type of infection which developed in the vampire bat is a blind end in the ecology of the virus in that it is not perpetuated by the animal infected by bat-bite, i e, they develop the paralytic form of the disease and do not become vicious and bite Therefore, the virus may have a cycle of infection which is like that of the true natural host where the perpetuation of the virus does not depend on the development of encephalitis in the infected animal, for instance, transmission by a cycle involving the infection in the breast tissue or the kidney One can assume that infection by bite in fighting among bats would not be so apt to produce the nonencephalitic type of infection noted in the vampire bat

The ecology of weasels of the *Mustela* genus is not so very different from that of the vampire bat The weasel exhibits a preference for feeding on blood They are good climbers and would likely prey on bats found in treeholes and on the branches of trees Vampire bats living in hollow trees would be likely prey for weasels, and colonial bats which live in caves would be taken by this animal as well There are many members of the families *Mustelidae* and *Viverridae* which have habits similar to that of the weasel in North America The small spotted skunk, *Spilogale putorius*, evidently plays an important part in the ecology of rabies virus in North America This skunk is a good climber and could thus serve to spread the infection to bats

The existence of rabies virus in the families *Mustelidae* and *Viverridae*, may be the equivalent of a built-in antibiotic against the carnivores of the family *Canidae* The virus does seem to be a major factor in controlling the population of wolves, foxes, jackals, coyotes and wild dogs There is the possibility that the virus is a natural disease of small mammals such as voles and bats, and that they perpetuate the virus during interepidemic periods, but this seems unlikely on the basis of present knowledge of the epidemiology of rabies

There has been a sharp reduction in the incidence of dog rabies in the United States during the past few years The enforcement of proper dog-control regulations and vaccination of domestic dogs with the very effective Flury LEP live-virus vaccine can be expected to eliminate dog rabies from any community within a few months The incidence of rabies in man has been reduced also, for example, in 1908 there were 111 human rabies cases in the United States (Stimson, 1910) compared with only 5 cases of rabies in man recorded in 1955 (Chadwick, 1956)

The attack rate of rabies in human beings bitten by rabid animals depends on several factors, for example, a rabid animal may not have the virus in its salivary glands, protection provided by clothing may be so great that little or no virus enters the wound produced, the natural source of the virus may be such that it is not very infectious for man, and virus may be removed from open wounds by cleansing with soap and water Nevertheless, the susceptibility of man to canine rabies appears to be relatively great Fetherston and Cooper (1932) observed 4 cases of rabies in 8 sailors bitten on their hands by a stray dog which they had picked up in a Chinese port and taken on board a destroyer, the bites of wolves are particularly dangerous as shown by the statistics given by Baltazard (1954), who recorded 60 rabies deaths, a mortality rate of 18 per cent, among 325 persons bitten by rabid wolves and given postexposure vaccine, and 53 rabies deaths, a mortality rate of 28 per cent, among 186 persons bitten on the head, despite vaccine administration Veeraraghavan (1954) arrived at a mortality rate by including only those bitten by an animal proved to have transmitted rabies to persons or animals In this category he recorded 19 rabies cases, a rate of 43 per cent, among 44 persons that did not receive the vaccine, and 10 rabies cases, a rate of 8 per cent, among 120 persons receiving a full course of vaccine The most extensive statistics as to attack rate of rabies in man are those of Schuder which as quoted by Kraus et al (1926), list 1,325 rabies deaths in a group of 14,959 persons bitten by rabid animals, a rate of 9 per cent This information was obtained from records antedating vaccine prevention Of the first 214 patients with face bites treated at the Pasteur Institute in Paris, 12 or 5 6 per cent died of rabies despite vaccine administration The danger of bites about the head and the face is great, as shown by the statistics reported by Kraus et al (1926), a mortality rate of 11 per cent for children and 3 per

cent for adults bitten on the head by animals suspected of being rabid, despite administration of vaccine A false sense of security is given by statistics on vaccination against rabies as given by rabies treatment centers, as they ordinarily include only those cases of rabies that develop 2 or more weeks after the completion of the treatment, the assumption being that those developing rabies before that time do not represent failures For example, McKendrick (1940) in his 9th review of the statistics on rabies vaccination gave a reduced fatality rate of 0 48 for 490,670 cases of rabies exposure given the Semple killed-virus vaccine It is statistics such as those given previously that emphasize the necessity of giving hyperimmune serum in addition to vaccine in all severe bite exposures to rabies

CONTROL

Measures necessary for the eradication of dog rabies have been known for more than 100 years, they are measures which prevent any dog from biting another for a period of the longest latency of the disease The development of an effective live-virus vaccine for rabies has simplified the control of dog rabies The essential regulations include registration and licensing of dogs, seizure and destruction of stray dogs, and restraint of all owned dogs during the control program, which should include mass immunization of dogs with the Flury LEP modified rabies live-virus vaccine In order to ensure an effective control program there must be a uniform method for rabies control in the state jurisdiction and co-ordination of state programs through regional interstate organizations The public health officer responsible for the state rabies control program should be a veterinary medical doctor, on the staff of the communicable disease division of the state department of public health This official then is in a position to promote and develop rabies control programs in the counties when rabies infection is identified in animals A system of weekly reporting of clinical cases of rabies in animals is an essential step in a state rabies control program, and laboratory facilities must be available so that the existence of the disease in animals can be confirmed by laboratory studies prior to enforcement of control regulations The county rabies control program should be developed in co-operation with local agencies. A local rabies committee should be formed with representation including the local health officer, a physician, a veterinary medical doctor, the mayor of the city, the chairman of the board of supervisors, and representatives of the livestock, agricultural, dog-owner and animal-protection organizations, approved by the state health officer. This type of committee can arrange for the implementation of the recommended rabies control procedures which must be done by the city council and the board of supervisors. Local government must provide dog-pound facilities, personnel and equipment for stray-dog control and arrange for vaccination of dogs. The dog license fee is the proper source of funds for stray-dog control The county rabies control program should be initiated promptly after the identification of rabies

The Flury LEP modified rabies live-virus vaccine is recommended for the immunization of dogs. There is no scientific basis for the continuation of the use of the killed-virus vaccine for immunization of dogs The Semple type killed-virus vaccine is a good antigen but it has the disadvantage of producing occasional cases of paralysis from sensitization to the mammalian brain tissue in the vaccine, and the immunity produced by the killed-virus vaccine is not so great or long lasting as that obtained with the live-virus vaccine. The production of rabies vaccine from virus cultivated in chick embryos has the advantage that the virus can be obtained free of bacterial contamination, and it can be preserved by lyophilization so that the keeping qualities are much better than that of the killed-virus product Many commercial biologic laboratories now produce the Flury LEP modified live-virus vaccine, and the seed virus is available for use in governmental or commercial laboratories in other countries The experimental studies and field trials done some years ago with the Flury LEP live-virus vaccine showed that it was antigenic and that dogs immunized with the vaccine virus showed no serious reactions attributable to the virus in the vaccine (Koprowski, 1949; Cox, 1949; Tierkel et al, 1949) Subsequently, large-scale field trials were con-

ducted in the United States, Israel, Rhodesia and Malaya, and the use of the vaccine under field conditions confirmed the early reports as to safety and effectiveness (Kaplan et al, 1954, Adamson, 1954, Wells, 1954, Starr et al, 1956) The demonstration that a single intramuscular injection of 3 cc of the Flury LEP live-virus vaccine would produce 100 per cent immunity to street virus in dogs for 3 years, under experimental conditions (Tierkel et al, 1953), furnished scientific evidence of the superiority of this vaccine to killed-virus vaccine Several million dogs have now been immunized with the Flury LEP live-virus vaccine. Reactions reported following vaccination have been accounted for by factors other than the presence of the Flury LEP virus in the vaccine, for example, the occurrence of epidemic diseases other than rabies, such as canine infectious hepatitis and distemper, in dogs at the time of the vaccination program The Flury LEP virus does not invade the salivary glands, so there is no danger of spread of the virus from the saliva of vaccinated dogs

If rabies [redacted] present in dogs or cattle when a vaccination program is initiated, one can expect the occurrence of rabies in vaccinated animals for at least 1 month after vaccination, as the result of exposure to rabies prior to vaccination The postexposure vaccine protection effect was demonstrated in dogs immunized with a single injection of Flury LEP live-virus vaccine in Malaya, in that rabies deaths were not observed in vaccinated dogs more than 1 month after vaccination, although cases of rabies continued to occur in unvaccinated dogs (Westgarth and Wells, 1957) An epidemic of cattle rabies was reported by Wilcox and Hubbard (1957) where 24 of a herd of 62 cattle died of rabies at one dairy, without a definite history of animal-bite exposure A stray dog had been seen at the dairy shortly before the onset of the epidemic The cattle were not vaccinated after the discovery of the disease Vaccination of this herd after the appearance of rabies would have resulted in many apparent failures, or the rabies cases observed following vaccination might have been attributed to infection from vaccination This type of rabies outbreak in cattle may explain the results observed in one herd of cattle vaccinated with Flury LEP live-virus vaccine, reported by Starr et al (1956), and the cases of rabies attributed to the use of this vaccine in Honduras, reported by Schroeder et al (1952), where the vaccine was tested in cattle during an outbreak of paralytic rabies, presumably of vampire bat origin The known high susceptibility of cattle to rabies and the development of the Flury HEP virus strain of low pathogenicity for mammals prompted the testing of this virus in cattle, and it was found to produce a satisfactory immunity to street virus (Koprowski and Black, 1954b, Starr et al, 1956) The Flury HEP live-virus vaccine has been used for the immunization of more than 2 million head of cattle in Mexico, and it has had extensive field trial in Brazil and the United States (Camargo, 1955, Carneiro, 1954, Starr et al, 1956). There have been no serious reactions to the vaccine, and a single intramuscular injection of the vaccine has been found to be effective in preventing rabies under field conditions The Flury LEP live-virus vaccine has been found to be satisfactory for the immunization of domestic cats (Cox, 1953). The dosage recommended is 1 5 cc given by intramuscular injection into the thigh muscle The use of the Flury HEP live-virus vaccine for immunization of dogs is not indicated until satisfactory evidence is obtained that the immunity produced is equal to that obtained with the Flury LEP virus

Dog vaccination programs should provide for the immunization of all owned dogs, 4 months or older, with a single intramuscular injection of 3 cc. of the Flury LEP live-virus vaccine, given in the thigh muscle The vaccinated dogs must not be allowed to run at large until 30 days have elapsed after vaccination The duration of immunity can be expected to be at least 3 years, and revaccination need not be required within this period of time in a dog rabies control program Dogs under 4 months of age are to be confined at all times in an area declared as infected with rabies When rabies is present in a community, any dog that has bitten a person must be confined in a veterinary hospital or dog pound for at least 7 days to allow for observation for signs of rabies and to ensure that the animal does not escape This is very important because of the great

number of dog bites observed in urban communities If the biting dog disappears, one must begin rabies vaccine administration immediately, because there is no way to determine that the dog did not have rabies When a rabid dog is discovered, every effort should be made to locate all other animals that were exposed to it so that they may be destroyed or vaccinated and kept in quarantine for 90 days For dogs previously vaccinated with the Flury live-virus vaccine, revaccination is recommended, and the quarantine period may be reduced to 30 days

Epidemic rabies in wild animals, such as wolves, foxes, coyotes, jackals and skunks, may be combatted by hunting and trapping. The U S Fish and Wildlife Service has trained personnel to supervise this type of work The use of poison-bait is effective but must be done by trained personnel If the hosts of the epidemic disease in wild animals are reduced drastically in number in and around a focus of rabies, the disease may be controlled quickly in this area (Johnson, 1945a) If this is not done, the disease will kill most of the animals anyway, and in the meantime the infection will have spread to other regions. The endemic foci of rabies have not been defined for lack of knowledge of the nature of the infection in the reservoir host When this is determined, methods can be developed to prevent the spread of the disease from such foci. It is inexcusable for any community to permit the existence of dog rabies and so endanger the public to this terrible disease

REFERENCES

(For complete references, see also Chapter 12, second edition)

Adamson, J S, 1954, Ecology of rabies in southern Rhodesia, Bull World Health Organ 10, 753-759.

Atanasiu, P, et al, 1956, Rabies neutralizing antibody response to different schedules of serum and vaccine inoculations in non-exposed persons, Bull World Health Organ 14, 593-611

Baltazard, M, and Bahmanyar, M, 1955, Essai pratique du sérum antirabique chez les mordus par loups enragés, Bull World Health Organ 13, 747-772

Bernkopf, H, and Kligler, I J, 1940, Characteristics of a fixed rabies virus cultivated on developing chick embryos, Proc Soc Exper Biol & Med 45, 332-335

Burns, K. F, Farinacci, C. F, Murnane, T. G, and Shelton, D F, 1956, Insectivorous bats naturally infected with rabies in Southwestern United States, Am J Pub Health 46, 1089-1097

Camargo, F, 1955, The derriengue problem in Mexico Proc 59th Annual Meeting U S Livestock San Assn, pp 313-318

Carini, A, 1911, Sur une grande épizootie de rage, Ann Inst Pasteur 25, 843-846

Carneiro, V, 1954, Transmission of rabies by bats in Latin America, Bull World Health Organ 10, 775-780

Chadwick, V D (Chairman, Committee), 1954, Report of the committee on rabies Proc 58th Annual Meeting U S Livestock San Assn, pp 357-362

———, 1956, Report of committee on rabies Proc 60th Annual Meeting U S Livestock San Assn, pp 256-262

Cox, H R, 1949, Review of chick-embryo adapted

—

Annual Meeting U S Livestock San Assn, pp 305-313

Cox, H R, Van der Scheer, J, Aiston, S, and Bohnel, E, 1947, The purification and concentration of influenza-virus by means of alcohol precipitation, J Immunol 56, 149-166

Delville, J P, and Jezierski, A, 1950, Isolement à partir d'un cerveau de chien d'un virus neurotrope pathogène pour de nombreuses espèces animales, Ann Soc Belge Méd Trop 30, 405-409

Enright, J B, 1956, Bats, and their relation to rabies, Ann Rev Microbiol 10, 369-392

Ferenbaugh, T L, 1916, A note concerning the occurrence of hydrophobia in the foxes of Alaska, Mil Surgeon 38, 656-657.

Fetherston, J E, and Cooper, G F, 1932, Rabies Report of four deaths resulting from bites on the fingers by the same rabid animal, U S Naval M Bull 30, 314-321

Fox, J P, Koprowski, H, Conwell, D P, Black, L, and Gelfand, H M, 1957, Study of antirabies immunization of man Observations with HEP Flury and other vaccines, with and without hyperimmune serum, in primary and recall immunizations, Bull. World Health Organ 17, 869-904

Galloway, I A, and Elford, W J, 1936, Size of virus of rabies ("fixed" strain) by ultrafiltration analysis, J Hyg 36, 532-535

Geiger, J C, 1916, Is rabies under control in California? California State J Med 14, 58-60

Giron, A T, 1944, El vampiro portador de virus del derriengue, Rev Soc Mexicana Hist Natural 5, 35-42.

Habel, K, 1945, Seroprophylaxis in experimental rabies, Pub Health Rep 60, 545-560

———, 1954 Habel test for potency in Laboratory

Techniques in Rabies, WHO Monograph Ser., No 23, 112-116

Harris, D L, and Shackell, L F., 1955, The effect of vacuum desiccation on the virus of rabies, with remarks on a new method, J Infect Dis 8, 47-49

Haupt, H, and Rehaag, H, 1921, Durch Fledermäuse verbreitete seuchenhafte Tollwut unter Viehbestanden in Santa Catharina (Sud-Brasilien) Ztschr Infektionskr 22, 104-127

Hodes, H L, Webster, L T, and Lavin, G I, 1940, The use of ultraviolet light in preparing a non-virulent antirabies vaccine, J Exper Med 72, 437-444

Horack, H M, 1939, Allergy as a factor in the development of reactions to antirabic treatment, Am J M Sc 197, 672-681

Hoyt, A, and Gutley, M K, 1938, Experimental street virus rabies in white mice Studies on passive immunization II Proc Soc Exper Biol & Med 38, 40-43

Hoyt, A, and Jungeblut, C W, 1930, Experimental rabies in white mice and attempted chemotherapy, J Infect Dis 47, 418-424

Hudson, J R, 1944, A short note on the history of rabies in Kenya, East African M J 21, 322-327

Hurst, E W, and Pawan, J L, 1931, An outbreak of rabies in Trinidad, Lancet 2, 622-628

———, 1932, A further account of the Trinidad outbreak of acute rabic myelitis, J Path & Bact 35, 301-321

Johnson, H N, 1945a, Fox rabies, J M A Alabama 14, 268-271

———, 1945b, Experimental and field studies of canine rabies vaccination Proc 49th Annual Meeting U S Livestock San Assn, pp 99-107

———, 1948, Derriengue, vampire bat rabies in Mexico, Am J Hyg 47, 189-204

———, 1954, Experimental studies on the duration of immunity in dogs vaccinated against rabies, Bull World Health Organ 10, 725 729

———, 1956, Rabies in Diagnostic Procedures for Virus and Rickettsial Diseases, pp 207-240, New York, Am Pub Health A

Kabat, E A, Wolf, A, and Bezer, A E, 1947, The rapid production of acute disseminated encephalomyelitis in rhesus monkeys by injection of heterologous and homologous brain tissue with adjuvants, J Exper Med 85, 117-130

Kanazawa, K, 1936, Sur la culture in vitro du virus de la rage, Jap J Exper Med 14, 519-522

Kaplan, M M, 1954, Potency-test requirements of United States National Institutes of Health (NIH) in Laboratory Techniques in Rabies, WHO Monograph Ser, No 23, pp 117-124

Kaplan, M M, Goor, Y, and Tierkel, E S, 1954, A field demonstration of rabies control using chicken-embryo vaccine in dogs, Bull World Health Organ 10, 743-752

Kissling, R E, 1958, Growth of rabies virus in nonnervous tissue culture, Proc Soc Exper Biol & Med 98, 223-225

Kligler, I J, and Bernkopf, H, 1938, Cultivation of rabies virus in the allantois of the developing chick embryo, Proc Soc Exper Biol & Med 39, 212-214

Koch, J, 1930, Lyssa in Kolle, W, Kraus, R, and Uhlenhuth, P Handbuch der pathogenen Mikroorganismen, ed 3, vol 8, pp 547-673, Jena, Fischer

Koprowski, H, 1949, Experimental studies of rabies virus, Canad J Pub Health 40, 60 67

———, 1954a, Potency test for chicken-embryo vaccine in Laboratory Techniques in Rabies, WHO Monograph Ser, No 23, pp 128-132

———, 1954b, Biological modification of rabies virus as a result of its adaptation to chicks and developing chick embryos, Bull World Health Organ 10, 709-724

Koprowski, H, and Black, J, 1952, Studies on chick embryo adapted rabies virus III Duration of immunity in vaccinated dogs Proc Soc Exper Biol & Med 80, 410-415

———, 1954b, Studies on chick-embryo-adapted rabies virus VII Immunological responses of animals to vaccination with high egg passage Flury strain J Immunol 72, 503-510

Koprowski, H, Black, J, and Nelsen, D J, 1954a, Studies on chick-embryo-adapted-rabies virus VI Further changes in pathogenic properties following prolonged cultivation in the developing chick embryo J Immunol 72, 94-106

Koprowski, H, and Cox, H R, 1948a, Studies on chick embryo adapted rabies virus I Culture characteristics and pathogenicity J Immunol 60, 533-554

———, 1948b, Occurrence of rabies virus in the blood of developing chick embryo, Proc Soc Exper Biol & Med 68, 612-615

Koprowski, H, Scheer, J van der, and Black, J, 1950, Use of hyperimmune antirabies serum concentrates in experimental rabies, Am J Med 8, 412-420

Korns, R F, and Zeissig, A, 1948, Dog, fox, and cattle rabies in New York State evaluation of vaccination of dogs, Am J Pub Health 38, 50-65

Kraus, R, Gerlach, F, and Schweinburg, F, 1926, Lyssa bei Mensch und Tier, Berlin, Urban

McKendrick, A G, 1933, Rabies a review of recent articles, Trop Dis Bull 30, 575-587

———, 1940, A ninth analytical review of reports from Pasteur Institutes on the results of anti-rabies treatment Bull Health Organ, League of Nations 9, 31-78

Mallory, F B, 1938, Pathological Technique, Philadelphia, Saunders

Mallory, L B, 1915, Campaign against rabies in Modoc and Lassen counties, California California State Board Health Bull 11, 273-277

Morgan, I M, 1947, Allergic encephalomyelitis in monkeys in response to injection of normal monkey nervous tissue, J Exper Med 85, 131-140

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REFERENCES

(For complete references, see also Chapter 12, second edition)

Adamson, J. S , 1954, Ecology of rabies in southern Rhodesia, Bull World Health Organ 10, 753-759

Atanasiu, P , et al , 1956, Rabies neutralizing antibody response to different schedules of serum and vaccine inoculations in non-exposed persons, Bull World Health Organ 14, 593-611

Baltazard, M , and Bahmanyar, M , 1955, Essai pratique du sérum antirabique chez les mordus par loups enragés, Bull World Health Organ 13, 747-772

Bernkopf, H , and Kligler, I J , 1940, Characteristics of a fixed rabies virus cultivated on developing chick embryos, Proc. Soc Exper Biol & Med 45, 332-335

Burns, K. F., Farinacci, C. F , Murnane, T. G , and Shelton, D F , 1956, Insectivorous bats naturally infected with rabies in Southwestern United States, Am J Pub Health 46, 1089-1097

Camargo, F , 1955, The derriengue problem in Mexico Proc 59th Annual Meeting U S Livestock San Assn , pp 313-318

Carini, A , 1911, Sur une grande epizootie de rage, Ann Inst Pasteur 25, 843-846

Carneiro, V , 1954, Transmission of rabies by bats in Latin America, Bull. World Health Organ 10, 775-780

Chadwick, V D (Chairman, Committee), 1954, Report of the committee on rabies Proc 58th Annual Meeting U S Livestock San Assn , pp 357-362

———, 1956, Report of committee on rabies Proc 60th Annual Meeting U S Livestock San Assn , pp 256-262

Cox, H R , 1949, Review of chick-embryo adapted living rabies virus vaccines, with primary emphasis on use in dogs Proc 53rd Annual Meeting U S Livestock San Assn , pp 264-272

———, 1953, Avianized rabies vaccine Proc 57th Annual Meeting U S Livestock San Assn , pp 305-313

Cox, H R , Van der Scheer, J , Aiston, S , and Bohnel, E , 1947, The purification and concentration of influenza-virus by means of alcohol precipitation, J Immunol 56, 149-166.

Delville, J P , and Jezierski, A , 1950, Isolement à partir d'un cerveau de chien d'un virus neurotrope pathogène pour de nombreuses especes animales, Ann Soc Belge Med Trop 30, 405-409

Enright, J B , 1956, Bats, and their relation to rabies, Ann Rev. Microbiol 10, 369-392.

Ferenbaugh, T L , 1916, A note concerning the occurrence of hydrophobia in the foxes of Alaska, Mil Surgeon 38, 656-657

Fetherston, J E , and Cooper, G F , 1932, Rabies Report of four deaths resulting from bites on the fingers by the same rabid animal, U S Naval M Bull 30, 314-321

Fox, J P , Koprowski, H , Conwell, D P , Black, L , and Gelfand, H M , 1957, Study of antirabies immunization of man Observations with HEP Flury and other vaccines, with and without hyperimmune serum, in primary and recall immunizations, Bull World Health Organ 17, 869-904

Galloway, I A , and Elford, W J , 1936, Size of virus of rabies ("fixed" strain) by ultrafiltration analysis, J Hyg 36, 532-535

Geiger, J C , 1916, Is rabies under control in California? California State J Med 14, 58-60

Giron, A T , 1944, El vampiro portador de virus del derriengue, Rev Soc Mexicana Hist Natural 5, 35-42.

Habel, K., 1945, Seroprophylaxis in experimental rabies, Pub Health Rep 60, 545-560

———, 1954 Habel test for potency in Laboratory

Wilcox, F P., and Hubbard, R C., 1957, Rabies epizootic in dairy cattle, J Am Vet M A *130*, 63-66
Wilkinson, D L., 1894, Rabies and hydrophobia in Alabama, Alabama Med [redacted] Surg Age, *6*, 557-584
Witte, E J., 1954, Bat rabies in Pennsylvania, Am J Pub Health *44*, 186-187
Wright, J T., and Habel, K., 1948, A comparison of antigenicity and certain biological characteristics of 6 substrains of Pasteur fixed rabies virus, J Immunol *60*, 503-515
Yoshino, K., Kuma, N., Kondo, A., and Kitaoka, M., 1956, Infection of the one-day old fertile hen's egg with rabies virus I Growth curves and serial passages Jap J M Sc & Biol *9*, 259-271
Yount, [redacted] E., 1910, Rabies, with reports of cases from skunk bites, South California Pract *25*, 105-116

Negri, A, 1903, Beitrag zum Studium der Aetiologie der Tollwuth, Ztschr Hyg 43, 507-528

Nelson, E W, 1918, Wild Animals of North America Nat Geograph Soc, Washington, D C., Judd & Detweiler

Nicolau, S, Mathis, C, and Constantinesco, V, 1933, La rage autochtone (maladie du chien fou) en Afrique occidentale française (etude critique et experimentale) Ann Inst Pasteur 50, 778-839

Pasteur, L, 1885, Méthode pour prévenir la rage après morsure, Compt rend Acad sc 101, 765-772

Pasteur, L, Chamberland and Roux, 1884, Nouvelle communication sur la rage, Compt rend Acad sc 98, 457-463

Pasteur, L, Chamberland, Roux, and Thuillier, 1881, Sur la rage, Compt rend Acad sc 95, 457-463

Pawan, J L, 1936a, The transmission of paralytic rabies in Trinidad by the vampire bat (*Desmodus rotundus murinus* Wagner, 1840), Ann Trop Med 30, 101-130

———, 1936b, Rabies in the vampire bat of Trinidad, with special reference to the clinical course and latency of infection, Ann Trop Med 30, 401-422

Plummer, P J G, 1954, Rabies in Canada, with special reference to wildlife reservoirs, Bull World Health Organ 10, 767-774

Proca, G, and Bobes, S, 1940, Anti-rabic immunisation living vaccines and killed vaccines, Bull Health Organ, League of Nations, 9, 79-130

Queiroz Lima, E de, 1934, A transmissao da Raiva bovina pelo morcego hematophago—*Desmodus rotundus*, Brasil Med 48, 38-40

Rivers, T M, Sprunt, D H, and Berry, G P., 1933, Observations on attempts to produce acute disseminated encephalomyelitis in monkeys, J Exper Med 58, 39-53

Schroeder, C R, Black, J, Burkhart, R L, and Koprowski, H, 1952, Rabies in cattle I Prevention of vampire bat paralytic rabies, derriengue, by vaccination with chick-embryo-adapted rabies virus, Vet Med 47, 502-506

Sellers, T F, 1927, A new method for staining Negri bodies of rabies, Am J Pub Health 17, 1080-1081

———, 1947, Complications of antirabic treatment, J M A Georgia 3o, 30-35

Seton, E T, 1925, Lives of Game Animals, Garden City, Doubleday, Volumes 1, 2

Sharpless, G R, Black, J, Cox, H R, and Ruegsegger, J M, 1957, Preliminary observations in primary antirabies immunization of man with different types of high-egg-passage Flury virus, Bull World Health Organ 17, 905-910

Shortt, H E, McGuire, J P., Brooks, A G, and Stephens, E D, 1935, Anti-rabic immunization Probable lines of progress in improvement of methods, Indian J M Res 22, 537-556

Snyman, P. S, 1937, Rabies in South Africa, J South African Vet M A 8, 126-133.

Starr, L E, Canup, S M, Watson, E L, and Jernigan, M, 1956, State-wide rabies control in Georgia, 1946-1956 Proc 60th Annual Meeting U S Livestock San Assn, pp 231-235.

Stimson, A M, 1910, Facts and Problems of Rabies, Government Printing Office, Washington, D C (Hyg Lab. Bull 65).

Stuart, G, and Krikorian, K S, 1928 The neuroparalytic accidents of anti-rabies treatment, Ann Trop. Med 22, 327-377

Sullivan, T. D, Grimes, J E, Eads, R. B., Menzies, G C, and Irons, J V, 1954, Recovery of rabies virus from colonial bats in Texas, Pub Health Rep 69, 766-768.

Thacher, J, 1812, Observations on Hydrophobia, Plymouth, Mass, Joseph Arey.

Tierkel, E S., Kissling, R E, Edison, M, and Habel, K, 1953, A brief survey and progress report of controlled comparative experiments in canine rabies immunization Proc 90th Annual Meeting, Am Vet Med Assn, pp 443-446

Tierkel, E S, Koprowski, H, Black, J, and Gorrie, R. H, 1949, Preliminary observations in the comparative prophylactic vaccination of dogs against rabies with living virus vaccines and phenolized vaccine, Am J Vet Res 10, 361-367

Umeno, W, and Doi, Y, 1921, A study on the antirabic inoculation of dogs, Kitasato Arch Exper Med 4, 89-108

Veeraraghavan, N, 1954, Phenolized vaccine treatment of people exposed to rabies in southern India, Bull World Health Organ 10, 789-796

———, 1956, Annual Report of the Director for 1954, Pasteur Instit southern India, Coonoor, Diocesan Press, Madras

Venters, H. D., Hoffert, W R, Scatterday, J E, and Hardy, A V, 1954, Rabies in bats in Florida, Am J Pub Health 44, 182-185.

Verteuil, E de, and Urich, F W, 1936, The study and control of paralytic rabies transmitted by bats in Trinidad, British West Indies, Trans Roy Soc Trop Med & Hyg 29, 317-347.

Villa, B, 1956, Tadarida brasiliensis mexicana (Saussure), el murcielago guanero, es una subespecie migratoria Acta Zoologica Mexicana 1, 1-11

Webster, L T, 1936, Diagnostic and immunological tests of rabies in mice Am. J Pub Health 26, 1207-1210

———, 1939, A mouse test for measuring the immunizing potency of antirabies vaccines, J. Exper. Med 70, 87-106

Webster, L. T, and Clow, A D, 1936, Propagation of rabies virus in tissue culture and the successful use of culture virus as an antirabic vaccine, Science 84, 487-488

Webster, L T, and Dawson, J R, 1935, Early diagnosis of rabies by mouse inoculation Measurement of humoral immunity to rabies by mouse protection test Proc Soc Exper Biol & Med 32, 570-573

Wells, C W, 1954, The control of rabies in Malaya through compulsory mass vaccination of dogs, Bull World Health Organ 10, 731-742.

Westgarth, D R, and Wells, C. W, 1957, The apparent inhibitory effect of avianized vaccine on canine rabies in the incubative stage, J. Am Vet M A 130, 57-60.

entists and the public through the medium of the National Foundation for Infantile Paralysis Never before has such extensive human experimentation been accepted by an informed public as occurred in the effort to control poliomyelitis.

Poliomyelitis is an acute febrile infection which causes as its most important result an inflammatory reaction in the central nervous system, producing in a variable proportion of cases a flaccid paralysis of irregular distribution, extent and permanence. The disease is caused by a small virus which very commonly infects the human alimentary tract rather generally all over the world. Usually, this infection causes no signs or symptoms of disease, though it may give rise to a minor illness which may be called "summer cold" or "intestinal flu" but cannot be accurately differentiated from any other trivial illnesses or even be recognized as an entity. In such instances, the virus grows harmlessly in the alimentary tract with occasional sorties into the blood stream or certain lymphatic channels The infection would be quite unimportant except that rarely, in relation to the total number of infected individuals, it invades the central nervous system by a route as yet imperfectly known and causes paralytic disease as the result of destruction of motor neurons in the medulla and the spinal cord

HISTORY

Poliomyelitis probably has been prevalent for a great many years, and in descriptions or old pictures of its disastrous results there are hints of its occurrence in periods that antedate the Christian era Only in the last 75 years, however, has it appeared in epidemic form

The name, acute anterior poliomyelitis, describes the very specific anatomic localization of the most characteristic pathologic changes due to this disease, the unique tendency of the virus to attack the anterior part of the gray matter of the spinal cord The disease is commonly known in English-speaking countries as infantile paralysis, a name well established by its original habits and general usage but misleading in that it implies a present predilection of the disease for infants Though it was primarily an infantile disease many years ago, it is not so in modern times and in countries where the disease is now most prevalent. It may still be considered an infantile disease at present in countries with poor sanitation, but it is also a rare and nonepidemic disease in these areas. The change in age incidence in civilized countries is of very great theoretic importance and will be discussed later.

Although this disease has been known since antiquity, one of the first accurate descriptions of some scattered sporadic cases was by Heine in 1840. For the next 50 years infantile paralysis continued to be a sort of medical curiosity which was frequently recorded in the literature. The disease also bears the name of Medin, 1890, since he apparently was the first to note an epidemic of any magnitude (44 cases in Stockholm during the summer of 1887). A little later Caverly, 1894, recorded a similar epidemic of 132 cases in Vermont, U.S.A But one of the early masterpieces of epidemiology was a monograph by Ivar Wickman, 1913, in which he analyzed the unprecedentedly large epidemic of 1905 in Sweden (1,031 cases). Although ignorant of the causal agent, Wickman was the first to present evidence for the transmission of the disease from person to person. Meanwhile, before Wickman's work appeared in English, Landsteiner and Popper, 1909, were able to show that a bacteria-free emulsion of spinal cord from a fatal human paralytic case reproduced the paralysis in monkeys within 7 to 14 days after it was injected into their brains This agent was soon shown to be a virus, and the foundation for modern research was laid.

CLINICAL PICTURE

TERMINOLOGY

It is necessary to define several terms which are used commonly in discussing poliomyelitis

Infection and Nonapparent Disease. Most poliomyelitic infections produce no signs or symptoms whatever and can be established only in the laboratory (Schabel et al, 1950, Bodian and Paffenbarger, 1954, Bhatt et al, 1955, Howe, 1958). This applies in almost equal degree to the identification of "abortive" poliomyelitic infection or "summer grippe" which almost certainly spares the central nervous system But the name may be changed to the "prodrome," the "first illness," or the "minor illness" if paralysis later ensues (Horstmann, 1949) In

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22

Poliomyelitis

(SYNONYMS. Acute anterior poliomyelitis, infantile paralysis, Heine-Medin disease, *Kinderlahmung, poliomyélite*)

INTRODUCTION

When the previous edition of this book appeared in 1952 the control of paralytic poliomyelitis was still in the blueprint stage. Now, a great reduction of incidence and possibly even eradication of the disease is feasible in many countries of the world

The unfolding story of this successful enterprise is one of the most dramatic in modern medicine. The success is especially a tribute to the importance of basic research in contributing new methods for approaching a chronically baffling problem The most important part of the story is the appearance of a new type of co-operative effort between sci-

EDITOR'S NOTE ENTEROVIRUSES. The 3 poliomyelitis chapters and the 2 chapters that follow are devoted to common virus diseases of late summer that are due to small viruses readily isolated from fecal specimens The chapters are arranged in order of seniority and the relative importance of the diseases The polioviruses are distinguished by their morbid effects in man and monkeys, the Coxsackie viruses by the lesions they induce in mice and the striking resistance to infection that mice quickly acquire after birth The ECHO viruses have no established pathogenicity for experimental animals They are cytopathogenic enteric viruses, without the characteristics of polio or Coxsackie viruses, that may be cultivated on human or monkey cells in vitro and are unrelated to other established viruses that, at times, may be recovered from the intestinal tract

A provisional classification and identification of the ECHO viruses was undertaken in 1955 by a committee of the National Foundation for Infantile Paralysis, and now 20 ECHO viruses have been recognized and identified by Arabic numerals The Foundation has also prepared typing sera to assist investigators in the identification of polio, Coxsackie and ECHO viruses The committee has suggested that the 3 groups be considered as members of a family of ENTEROVIRUSES (Committee on ECHO Viruses, Science, 1955, *122*, 1187-1188) This is consistent with the proposal of a Family of ENTEROVIRACEAE of the Order VIRALES which would include a Tribe of PARVOVIRAE (Dalldorf, G, Annals N Y Acad Sc 1953, *56*, 583-586) The 3 groups could properly be considered Genera within that Tribe

These suggestions will doubtless be reviewed by

among strains of the same antigenic type have made the characterization of certain types difficult Strain differences are reflected in striking variations in pathogenicity for both animals and cultured cells For example, the original strains of ECHO 9, isolated from healthy children, were devoid of pathogenicity for mice, while the epidemic strains of 1955 and 1956 were pathogenic for these animals and were recognized as Group A Coxsackie viruses This virus has now been properly designated a Group A virus (type 23) (Sickles, [redacted] M., in preparation) Other ECHO viruses may be reassigned after their properties are fully recognized

The growing realization that a number of Coxsackie and ECHO viruses are capable of inducing paralysis in man and poliomyelitislike lesions in monkeys provides additional justification for considering the 3 groups to be related and deserving of the attention that the several ENTEROVIRUSES are now receiving

entists and the public through the medium of the National Foundation for Infantile Paralysis. Never before has such extensive human experimentation been accepted by an informed public as occurred in the effort to control poliomyelitis.

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Terminology

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this uncertain state, the accuracy of guesses is considerably increased but by no means made certain if a mild febrile illness is detected in an epidemic setting characterized by frank paralysis or other signs of central nervous system invasion in other members of the family or close associates. Unfortunately, the most accurate method of diagnosis is retrospective, namely, a 4-fold or greater rise in the titer of specific serum antibody against some type of poliovirus Two bleedings must be obtained spanning an interval of 2 to 4 weeks While the isolation and the typing of poliovirus from the patient usually indicates the etiology, it is not positive proof, since occasionally a single individual may harbor more than one virus which is capable of evoking disease (Rhodes et al. 1950, Melnick et al, 1951, see section on Diagnosis)

The "*preparalytic stage*" of the disease is obviously the course up to the point of recognition of paralysis In those instances in which paralysis does not occur, this term is not applicable, we speak then of a nonparalytic course of the disease

"Bulbar poliomyel...
tinction
part of
volved, as judged by the clinical symptoms The term, though a poor one, is established in medical literature and refers to the type of disease that attacks predominantly the medulla or bulb of the brain and manifests itself by disturbances of the respiratory or vasomotor centers and by paralysis of the palate, the pharynx and the larynx The term is often used inaccurately for paralysis of muscles deriving their nerve supply from above the medulla, such as oculomotor and facial paralysis. The term is quite misused when it refers to respiratory paralysis due to involvement of the intercostal and diaphragmatic muscles We use it in this chapter in the usual though inexact sense, referring to brain-stem involvement It certainly would be clearer and more practical to refer only to the paralyzed muscle groups as pal-

...that it refers to a mild nondescript illness which may occur without any central nervous system symptoms and can be presumed to be due to the virus of poliomyelitis only because it occurs in members of a family coincidentally with an acute and definite case of poliomyelitis. It may well be associated with a viremia which does not lead to central nervous system involvement, but this has not been proved. Others use the term "abortive poliomyelitis" for the nonparalytic form of the central nervous system disease diagnosed by the clinical features and by the characteristic changes in the spinal fluid We use the term in the first sense.

The encephalitic type of the disease is a term used loosely when the patient's sensorium is particularly clouded

The Incubation Period

The incubation period of paralytic poliomyelitis is not definitely known Even during epidemic times, the presence of many asymptomatic alimentary infections, together with the protracted and frequently innocent appearing onset of paralytic disease in the primary case, has made it very difficult to determine accurately the time of effective exposure in those cases believed to be secondary

Casey (1942a, b) had a rare opportunity to study 36 cases (29 of them paralytic) which he had reason to believe were infected by a single exposure to a primary case They originated in a widely scattered rural population living in rough hilly country along "roads" which followed stream beds into blind ends without cross connections Opportunities for contact were rare and had fostered a local custom of elaborating a business transaction into a social event which involved two entire families spending the day together In this carefully scrutinized group, Casey found that the intervals from presumed exposure to the first symptoms ranged from 5 to 35 days and to paralysis 8 to 36 days, the latter averaging 17 days. In 14 highly susceptible chimpanzees fed virus and subsequently paralyzed, the time of exposure was more definitely known, and the incubation period to initial paralysis ranged from 7 to 31 days, with an average of 16 days (personal observations, H. H).

Outline of Clinical Course

The clinical course is as definite and points as dependably to a diagnosis as does the clinical picture of most other disease entities This statement should not imply that there are not frequent variations The clinical pic-

ture is studied best in the light of the usual sequence of events, which divides itself quite naturally into definite periods, and the differential diagnosis is discussed best with different emphasis for each period at which the disease may first be encountered Figure 62 in Chapter 23 shows the course graphically. It also shows the relationship of the finding of the virus and the occurrence of antibodies to the clinical features of the disease

It is always worthwhile to plot carefully in one's mind the time of onset and sequence of symptoms. Acute poliomyelitis is a disease of short duration, and its course does not vary much The ordinary febrile course consists of a rise of temperature associated with meningeal signs with or without paralysis, lasting a total of 5 to 7 days

Prodromal Stage A certain proportion of the patients show a preliminary rise of temperature for 2 or 3 days associated with no specific symptoms; this may be called a prodromal febrile stage and is followed by a period of well-being before onset of the ordinary febrile course It is quite possible for poliomyelitis to occur without any recognized febrile course, though any such instance should be studied with reasonable skepticism

The first hump of the temperature just referred to as a prodromal febrile stage (cf Fig 62, Chap 23) is made much of, but is seldom of aid in diagnosis. It is probably emphasized too greatly for the good of students, as it occurs in as little as 25 per cent of patients, the frequency probably depending on the acuteness of the observation of the family or the physician When the initial rise of temperature does occur, the resulting 2-humped temperature curve is termed the "dromedary course," this expression having been used by someone who thought the 2 temperature peaks resembled the humps of a camel It is unfortunate that the true dromedary has only 1 hump However, the term still persists

This prodromal or "dromedary" stage has acquired increasing interest with the knowledge that a viremia occurs, and many think that perhaps this represents the viremic stage of the disease This has not been shown by any adequate number of cases where the viremia was actually proved, and one can understand easily the difficulties of the situation since it is difficult to recognize until later that the illness is poliomyelitis.

The symptoms are nondescript during this period, and exact diagnosis at this time is impossible The spinal fluid is normal Suspicion of the disease can be aroused only because other members of the family or close associates are ill with infantile paralysis There may be slight fever and malaise, sometimes a headache or various gastro-intestinal disturbances, which ordinarily are passed off as a slight cold or "flu" or something equally trivial This first stage of the disease lasts from 1 to 2 days On the 4th to the 7th day following the first febrile rise, a second and definite abrupt rise of temperature takes place The same nondescript and mild symptoms may occur without being followed by any subsequent disease and constitute the "abortive poliomyelitis" referred to above

Preparalytic Stage. SYMPTOMS Three symptoms almost invariably occur at this time, i e, fever, vomiting and headache However, these symptoms are common to a great many diseases and in themselves do not lead to any particular diagnosis The fever is not high, usually under 103° F In the bulbar type of disease the fever is apt to be higher and to last over a longer period The vomiting is not like that caused by increased intracranial pressure and usually is not particularly forceful or projectile. It is associated with nausea. The headache is of moderate severity and constantly occurs though small children may not complain.

A variety of pains may occur, predominantly located in the back or the neck The neck pain often may not be reported as a "stiff" neck or back, and occasionally one gets a very illuminating story of a curious stiff gait possibly attributed to back sprain Usually the patient is sick enough to stay in bed, but it is not uncommon for him to remain ambulant up to the time of consulting a physician Although either diarrhea or constipation may occur, neither is common Sometimes constipation is very marked and seems definitely to be associated with atonia of the intestine Abdominal pain occasionally appears in the midepigastric region but is not ordinarily severe.

Physical Findings

The general appearance of the patient is of some diagnostic importance but actually cannot play a great part in establishing a diagnosis The patient is flushed and looks as if he had a temperature of 104° or 105° F., when actually his temperature is 101° or 102°, he is not greatly prostrated, is apt to be alert, co-operative, or even somewhat stimulated and talkative. The patient's skin is pink and flushed. A *tache cerebrale* which occurs also in many other diseases usually is demonstrated easily.

A positive tourniquet test or the easy appearance of petechiae on squeezing some part of the skin frequently can be demonstrated The pulse is fast, out of proportion to the actual fever and more in proportion to the appearance of the child Perspiration is marked and becomes more so as the disease progresses

Much emphasis must be put upon the detection of signs of central nervous system involvement, since it is by the demonstration of a characteristic type of stiff neck or back that a lumbar puncture is justified A dependable diagnosis of poliomyelitis in a preparalytic stage cannot be made without the demonstration of an abnormal spinal fluid

The rigidity of the neck and the back usually is not as marked as in meningitis and nowhere nearly as severe as in tetanus However, this varies and occasionally can be so striking that even parents notice it in their children and may report a curious, "stiff" type of gait The spinal rigidity is most marked in the cervical and the dorsal regions. It is demonstrated best, particularly in children, by asking the patient to sit up He may say he cannot, which may mean merely that he does not want to, though usually children will try If he co-operates a certain maneuver is characteristically carried out. He will turn on his side, push himself up on his hands and then turn around, sitting squarely on the bed but leaving both hands behind as a prop This is obviously to enable him to keep his balance without anterior flexion of the spine. If asked to put the chin on his sternum or on the examiner's finger put against the sternal notch, usually he will try to do so with careful, jerky, forward movements of his head and will open his mouth widely in an attempt to put his chin on the sternum with minimum flexing of the neck If a patient is lifted by his shoulders even with his co-operation, the head falls back, as if the neck were paralyzed. In fact, paralysis of the neck often has been wrongly diagnosed from this characteristic action. The patient allows the head to fall back not because it is paralyzed but because in that way painful flexion by his own muscle efforts is avoided. If the patient has any extensive neck muscle paralysis, he will not be able to turn his head from side to side, which he does fairly readily without pain when no paralysis exists.

A fine tremor often precedes by a few hours the development of paralysis. However, this should not be depended upon too greatly, because some patients will show quite marked tremors without subsequent paralysis developing. When it is seen localized in one extremity it can properly give the physician concern.

Paralytic Stage. It has been stated that paralysis may not occur. The temperature may become normal without any further symptoms. Muscle pain of considerable severity may occur, even if no paralysis is seen However, it is associated so often with paralysis that it is discussed in this section

Muscle pain varies considerably in individual patients and in different epidemics It is most marked during the early stage of paralysis and can be so severe and so localized as to imitate osteomyelitis or arthritis On the other hand, frequently such pain is absent. The presence of pain usually is evident from specific complaint by the patient; in young children or babies the fact that pain is present should be obvious from general observation while they are being handled.

Permanent damage to the central nervous system manifests itself almost exclusively by flaccid paralysis. Any other sequelae are hard to prove. Convulsions or upper motor neuron paralysis does not occur The disease does not affect permanently the sensory nerve cells, although it is possible that the pain of the acute stage of the disease is due to involvement of the posterior part of the cord. There is no disturbance of the sense of touch or pain Paralysis, when it occurs, usually will

be first evident on the 2nd, the 3rd or the 4th day after the first meningeal symptom Occasionally, paralysis will appear very rapidly in a few hours after the first sign of fever and occasionally as late as the 6th day. The peak of the incidence of paralysis is on the 3rd day. A reasonably good prognosis against the appearance of paralysis or an extension of it can be made by the time the temperature reaches normal, which is usually by the 5th or the 6th day, although it may be stated again that paralysis apparently due to poliomyelitis may occur without any recognized febrile illness

Because of pain or lack of co-operation of young patients, the certain detection of paralysis can be very difficult, and some reports of its extension late in the disease probably are due to earlier inaccurate observation Even a considerable degree of paralysis can be missed completely, particularly that of the paravertebral group of muscles and that occurring in young and un-co-operative children It is easy to understand that when pain or tenderness is marked, an evaluation of the extent of paralysis becomes difficult Although tenderness and paralysis often are associated, it does not certainly follow that a particularly tender muscle group will become paralyzed A physician often has the happy experience of finding that a tender muscle group, which he thought was paralyzed, shows good activity when the pain ceases Thus attempts to make an exact evaluation of paralysis are often futile in the acute stage of the disease when certain differentiation between inability and unwillingness to use a muscle is impossible

Characteristics of the Paralysis The paralysis of poliomyelitis is one of selected muscle groups It is not characteristic to have one extremity alone wholly paralyzed The paralysis in any muscle group can be of any degree, as it is obvious that a variable number of nerve cells can be destroyed Therefore, it is characteristic of poliomyelitis to cause a spotty paralysis, although in extensive involvement all the major muscle groups of an extremity may be completely lost Infantile [illegible]

of the paralysis does not follow the distribution of a nerve trunk such as the femoral or the glossopharyngeal nerve. Since a nerve trunk may derive its fibers from a fairly wide source in the cord or medulla, it is wiser to think of paralysis in terms of the muscles involved rather than of the nerve trunks supplying them. Large muscle groups of the extremities are paralyzed first and more extensively than the small muscles of the hands and the feet.

Detection of Paralysis The attempts at thorough muscle examination during the acute stage, especially when muscle tenderness exists, may be harmful as well as unsuccessful [illegible]

tion is necessary for prognosis as well as for planning treatment. An exact quantitative evaluation of the function of all muscle groups must remain the task of specially trained technicians, as over 100 separate muscle groups need to be tested and their power roughly quantitated However, the ordinary medical attendant should be able to detect paralysis adequately enough to give a dependable prognosis Careful observation of all spontaneous motions of an infant requires time but is of great value. For children a game can be invented easily whereby the examiner holds the child's arm or leg in some neutral position and then induces the small patient to resist motion in any direction

The physician always should be on the alert for the late discovery of paralysis originally missed Paralysis of the muscles of the trunk, particularly the abdominal muscles and the paravertebral groups, is apt to be overlooked and to lead to deforming scoliosis. Repeated muscle examinations should be carried out as carefully as possible at intervals of 2 or 3 months and then once again after 6 months or a year

"Bulbar" Type of Paralysis. The reported incidence of the bulbar form of the disease varies greatly as a physician's use of the term varies, but its incidence actually differs in different epidemics One case of bulbar paralysis for 8 or 10 cases of the spinal type is a reasonable expectation.

Much emphasis must be put upon the bulbar paralyses because of the high mortality in this group Paralysis of the face, the soft palate or

the pharynx—all called "bulbar" palsies—are frequently associated and are easy to recognize. Paralysis of the larynx is much more rare and is often difficult to detect clearly. Aphonia is uncommon but does occur A facial paralysis usually is associated with a palatal paralysis, and the latter with pharyngeal paralysis Either the palatal or the pharyngeal paralysis can occur alone Paralysis of the extrinsic muscles of the eyes is seen occasionally and usually is associated with one of the bulbar palsies

The recognition of a facial paralysis, which is, of course, of the central type, is easy Detection of palatal paralysis is made easy by the presence of a nasal voice and by the regurgitation of fluid through the nose on swallowing Pharyngeal paralysis likewise should be easy to diagnose but, nevertheless, resulting symptoms are frequently misinterpreted through inexperience Inability to swallow due to pharyngeal paralysis should be suspected and looked for whenever there is facial or palatal paralysis The first symptoms may be noisy, gurgling breathing and respiration which is irregular and interrupted The patient himself may not realize at first what the difficulty is, and the paralysis unfortunately may be demonstrated first by a severe choking attack following an attempt to drink Frequently, a doctor or a nurse may demonstrate the paralysis ill-advisedly by offering the patient a drink of water before the patient himself is aware of his disability. The patient may attempt to swallow a large mouthful: then he becomes frightened and begins to choke Sometimes in a paroxysm of fear, aspiration and laryngeal spasm may bring about severe anoxia with coma followed by a definite turn for the worse Great care should be exercised to avoid such episodes

Bulbar forms of poliomyelitis are associated with a longer and higher temperature course and with a more rapid pulse than in the usual spinal types of disease When the disease is fatal, frequently death is preceded by a period when the pulse is extraordinarily rapid, increasing hour by hour Rarely, there is a slow "vagal" pulse in acute bulbar poliomyelitis The blood pressure is not frequently elevated, but it may be so. It may also be greatly depressed Although the blood pressure may be elevated in a serious manner in later stages of the disease, it is interesting and puzzling that there is no necessary relationship between the two periods of hypertension Both hypertension and hypotension may occur with involvement of the medulla in the acute bulbar forms of poliomyelitis Hypertension occurs late in the course of this disease without any history that clearly points to brain-stem involvement at any stage The cause of this late hypertension is not clear. Renal stones are frequent in highly immobilized patients, but the occurrence of stones is not consistent with the hypertension The enormous reduction of the peripheral vascular bed with extreme muscular atrophy is also offered as an explanation of hypertension, but this seems inconclusive

LANDRYLIKE TYPE OF PARALYSIS Landry (1859) described a disease with paralysis progressing in the course of a few days from the feet and the legs to the trunk and upward to a fatal result with failure of the respiratory muscles or with bulbar symptoms He described also a descending paralysis with the same evidence of slow progression. Certainly, a picture like this occurs in epidemics of poliomyelitis and closely resembles the ordinary disease with pathologic findings similar to those of poliomyelitis

Encephalitic Type of Poliomyelitis. Although there is evidence from the pathology of the disease that poliomyelitis may attack any part of the central nervous system, clinical evidence of involvement of the higher brain centers is in most incidences meager. Ordinarily, the victim of poliomyelitis, except when *in extremis*, is mentally alert and shows none of the picture of encephalitis Sometimes, however, the sensorium is dull, there is excessive drowsiness, the patient is hard to arouse and presents a picture resembling that of tuberculous meningitis Except for this involvement of the general sensorium, we see little clinical evidence of encephalitis Intellectual defects as sequelae of poliomyelitis rarely, if ever, occur Upper-motor-neuron involvement producing a spastic paralysis must be extraordinarily unusual, although mentioned in early reports, and one should view with skepticism any such condition attributed to poliomyelitis Convulsions in poliomyelitis also are so rare that such a symptom should be considered as weighty evidence against

the diagnosis Many cases of encephalitis possibly caused by ■ related virus have been called poliomyelitis during epidemic times Modern virus studies do not support such diagnoses

Failure of Respiration. The final mechanism of death in any disease becomes almost a matter of philosophy. However, in poliomyelitis it seems more than usually logical to say that death is brought about by an immediate interference by the disease with the mechanism of respiration The analysis of such situations may by very difficult, but at some risk of oversimplication we can consider that efficient respiration may be prevented by 3 means

1 Disturbance of the nerve centers in the medulla or "bulb" which control respiration

2 The collection of mucus or vomitus around the glottis in patients with paralysis of the pharynx causing, either by actual obstruction or by setting up irritative spasms of the glottis, constantly interrupted inspiration and consequently shallow, irregular and ineffective respiratory efforts.

3 Actual paralysis of the primary respiratory muscles, the intercostals and the diaphragm

The lesions existing in the first and the second situations, involvement of the respiratory centers or paralysis of the pharynx, are in the medulla, therefore, this type of the disease commonly and properly is called "bulbar"

In the third situation, that with actual paralysis of the intercostal muscles or of the diaphragm, the lesion exists in the dorsal and the cervical sections of the cord Therefore, this spinal type of the disease should not be classed as "bulbar," although respiratory failure occurs

The respiratory difficulty in any patient ill with poliomyelitis may be due to one or to any combination of these 3 factors Paralysis of the respiratory muscles very frequently occurs alone without "bulbar" complications Paralysis of the pharynx, the palate or the facial muscles, all innervated from the medulla, very frequently is associated with apparent involvement of the "vital centers," most evidently the respiratory center. The marvelously complicated and congested mass of nerve paths and centers of the medulla makes it remarkable that such association does not always occur.

It is often difficult to ascertain the cause of the respiratory difficulty in the individual patient with "bulbar" poliomyelitis without paralysis of the intercostal muscles or of the diaphragm In some cases the respiratory disturbance seems to be purely "central" in origin and may make itself manifest by shallow irregular respirations or by jerky spasmodic inspiratory efforts, sometimes almost amounting to a succesion of hiccoughs

Pharyngeal Paralysis Pharyngeal paralysis alone can interfere with respiration, due to the existence of unswallowed pharyngeal secretions or vomitus which prevents the free passage of air through the larynx In pharyngeal paralysis often it is very difficult to be sure that there is not also some disturbance in the medulla of the nerve centers themselves controlling respiration It seems reasonable to hope that there is no "central" disturbance of respiration when the patient appears to be making conscious efforts to breathe carefully to clear his throat and seems to be alert to his own difficulties For many hours a patient may need consciously to plan every breath in a fatiguing effort to avoid aspiration Due to cumulative fatigue and anoxia, alert consciousness soon may be lost and be followed by a stage of semicoma with occasional periods of consciousness and fright, so that the condition is hard to distinguish from one due to a primary encephalopathy A very rapid pulse is characteristic of the bulbar form of poliomyelitis before death and is evidently a manifestation of the disturbance of the vagus nerve as well as the result of simple excitement and fatigue.

Respiratory Muscle Paralysis The early evidence of paralysis of the intercostal muscles or of the diaphragm may be difficult to recognize and to interpret Wakefulness, anxiety, restlessness, an increase ■ respiratory rate, the use of the alae nasi, a slight respiratory grunt, disinclinations to talk or a curious, frequently interrupted, monosyllabic speech all may precede more marked evidences of paralysis Cyanosis indicates severe paralysis and does not precede death by many hours.

In a child who will not co-operate by "taking a deep breath," it may be helpful in the demonstration of a partial paralysis of the

the pharynx—all called "bulbar" palsies—are frequently associated and are easy to recognize. Paralysis of the larynx is much more rare and is often difficult to detect clearly Aphonia is uncommon but does occur A facial paralysis usually is associated with a palatal paralysis, and the latter with pharyngeal paralysis. Either the palatal or the pharyngeal paralysis can occur alone Paralysis of the extrinsic muscles of the eyes is seen occasionally and usually is associated with one of the bulbar palsies

The recognition of a facial paralysis, which is, of course, of the central type, is easy. Detection of palatal paralysis is made easy by the presence of a nasal voice and by the regurgitation of fluid through the nose on swallowing Pharyngeal paralysis likewise should be easy to diagnose but, nevertheless, resulting symptoms are frequently misinterpreted through inexperience Inability to swallow due to pharyngeal paralysis should be suspected and looked for whenever there is facial or palatal paralysis The first symptoms may be noisy, gurgling breathing and respiration which is irregular and interrupted The patient himself may not realize at first what the difficulty is, and the paralysis unfortunately may be demonstrated first by a severe choking attack following an attempt to drink Frequently, a doctor or a nurse may demonstrate the paralysis ill-advisedly by offering the patient a drink of water before the patient himself is aware of his disability The patient may attempt to swallow a large mouthful, then he becomes frightened and begins to choke Sometimes in a paroxysm of fear, aspiration and laryngeal spasm may bring about severe anoxia with coma followed by a definite turn for the worse Great care should be exercised to avoid such episodes

Bulbar forms of poliomyelitis are associated with a longer and higher temperature course and with a more rapid pulse than in the usual spinal types of disease When the disease is fatal, frequently death is preceded by a period when the pulse is extraordinarily rapid, increasing hour by hour. Rarely, there is a slow "vagal" pulse in acute bulbar poliomyelitis The blood pressure is not frequently elevated, but it may be so. It may also be greatly depressed Although the blood pressure may be elevated in a serious manner in later stages of the disease, it is interesting and puzzling that there is no necessary relationship between the two periods of hypertension Both hypertension and hypotension may occur with involvement of the medulla in the acute bulbar forms of poliomyelitis Hypertension occurs late in the course of this disease without any history that clearly points to brain-stem involvement at any stage The cause of this late hypertension is not clear Renal stones are frequent in highly immobilized patients, but the occurrence of stones is not consistent with the hypertension The enormous reduction of the peripheral vascular bed with extreme muscular atrophy is also offered as an explanation of hypertension, but this seems inconclusive

Landrylike Type of Paralysis Landry (1859) described a disease with paralysis progressing in the course of a few days from the feet and the legs to the trunk and upward to a fatal result with failure of the respiratory muscles or with bulbar symptoms He described also a descending paralysis with the same evidence of slow progression Certainly, a picture like this occurs in epidemics of poliomyelitis and closely resembles the ordinary disease with pathologic findings similar to those of poliomyelitis

Encephalitic Type of Poliomyelitis. Although there is evidence from the pathology of the disease that poliomyelitis may attack any part of the central nervous system, clinical evidence of involvement of the higher brain centers is in most incidences meager. Ordinarily, the victim of poliomyelitis, except when *in extremis*, is mentally alert and shows none of the picture of encephalitis Sometimes, however, the sensorium is dull, there is excessive drowsiness, the patient is hard to arouse and presents a picture resembling that of tuberculous meningitis Except for this involvement of the general sensorium, we see little clinical evidence of encephalitis Intellectual defects as sequelae of poliomyelitis rarely, if ever, occur. Upper-motor-neuron involvement producing a spastic paralysis must be extraordinarily unusual, although mentioned in early reports, and one should view with skepticism any such condition attributed to poliomyelitis. Convulsions in poliomyelitis also are so rare that such a symptom should be considered as weighty evidence against

New tubes for collecting the fluid and new glass slides for examination of smears should be used Not infrequently, old and dead bacteria remaining on glassware from previous use may be stained again and cause serious confusion

SUGAR CONTENT The sugar content of the spinal fluid usually is normal or increased somewhat above average

EMOTIONAL DISTURBANCES IN POLIOMYELITIS

It is very evident that poliomyelitis is often accompanied by emotional disturbances which sometimes can be very severe Attention to these aspects of the disease has increased along with the greater interest in emotional disturbances that is now apparent in all medical practice In children this emotional disturbance may be seen, after the acute disease is past, by overactivity and incorrigibility (Coppelman, 1944) Many people are convinced that these changes in emotional reaction are more marked than after other acute infections

It has been questioned whether emotional disturbances are due to actual involvement of the brain by the virus of poliomyelitis It seems unlikely that this is so, since the virus does not directly attack any of the so-called "higher centers" outside the motor areas of the cortex, although at postmortem some histologic changes can be seen in all areas of the brain There are lesions in the thalamus, the subthalamus and the brain stem which conceivably could produce emotional instability Electroencephalographic studies (Holmgren, 1952) have been carried out in this disease (Grossman, et al, 1958, unpublished), and abnormalities have been found, but the electroencephalographic abnormalities seem to be found as frequently in patients without any of the so-called "encephalitic symptoms" EEG abnormalities have also been found in other acute diseases, for instance, in measles when there is no clinical evidence of encephalitis Therefore, the significance of these EEG findings must wait for more studies of many infectious diseases during the acute and the convalescent stages before conclusions regarding specificity can be drawn

Recently, as mentioned in the section on treatment, there has been a development of "Respirator Centers" where the worse handicapped victims of the disease—those with paralysis of respiratory muscles—are being treated very effectively and freed of their cumbersome assistive devices so they can return home Great attention has been paid to the emotional disturbance of these patients It would seem highly unlikely that psychic changes are more than might be expected in a highly publicized disease to which the public has become very sensitized and particularly in victims of the disease who themselves look with reasonable forebodings on their own future It would be expected that with the general knowledge of the crippling due to poliomyelitis, and the consequent increase and general fear of it, that a victim, knowing or suspecting his diagnosis, would fear the worst and that when actual paralysis does occur this fear would become greatly aggravated One vivid evidence of this with resulting physiologic disturbance is seen in patients with early respiratory muscle involvement Before they actually have severe paralysis, the patients overventilate and produce a mild alkalosis in themselves. This again might be a direct effect of the virus on the respiratory centers, although in all probability is not, but is simply another manifestation of a fear reaction This probability is more likely since similar hyperpnea is detected in patients with other respiratory handicaps such as asthma, as well as in patients frightened from other conditions than the disease itself. The emotional reactions take various forms, as they do in other diseases, attention to them, as phenomenon apart from virus effects, demands understanding care.

PROGNOSIS

General figures as to the fatality rate of poliomyelitis vary greatly (International Poliomyelitis Congresses, 1949, 1952, 1955) In New York in 1916 the fatality rate was reported as 21 4 per cent in 4,215 cases; in 1930 it was 16 8 per cent in 660 cases; in 1931 it was only 8 per cent in over 2,000 cases; in 1940 the United States fatality rate was about 10 per cent A reduction in reported fatality does not necessarily represent any lessening of the virulence of the disease but is in great part only evidence of an increasingly more astute diagnosis of the non-

muscles of respiration to inhibit the action, first of the intercostals, and then of the diaphragm, by splinting the chest or the abdomen with the hands and thus forcing the alternate respiratory muscles to greater action.

Postfebrile Course. After the fever has subsided, the clinical features of the postfebrile course of this disease are dominated by the picture of paralysis already described. However, certain other symptoms are evident, and it is clear that the disease attacks to some extent other parts of the central nervous system than the anterior horn cells. Vasomotor disturbances are evident during the acute stage of the disease, even though they are hard to define; they vary in intensity and might be considered no more prominent than in some diseases which do not attack the central nervous system Even without definite paralysis the patient is weak and unsteady as after any febrile illness. Complaints of easy fatigue, tachycardia, constipation, easy flushing and perspiration are made often and should be treated by rest and protection from overwork over a long period of convalescence

Clinical Laboratory Findings

Except from a study of the spinal fluid, the clinical laboratory has very little to offer of aid in the diagnosis or understanding of the course of poliomyelitis Poliomyelitis causes no change in the number or characteristics of red blood cells. Leukopenia is frequently seen early both in the human disease and in the experimental disease in monkeys However, there is not enough consistency in this finding to be of any dependable aid in differential diagnosis.

The spinal fluid is of great importance in the diagnosis of this disease during the acute febrile stage, although there are no changes in the fluid pathognomonic of poliomyelitis. One can say with some emphasis that, without characteristic paralysis, the diagnosis of

Characteristics of Spinal Fluid Globulin Content. The globulin content is increased fairly consistently. Statistically, the globulin is low at the early part of the disease, then rises fairly quickly and may be abnormally elevated for a considerable time after the end of the febrile period It is quite possible that the globulin test will be negative at the onset of the illness In the presence of an increase in the cell count of the spinal fluid the globulin test offers no additional information of value.

Cell Count. An increase in cells (normal; 0 to 8 per cu mm) appears within a few hours of the onset of fever and meningeal signs and usually persists during the febrile period. However, the cells may disappear in as short a period as 2 days or occasionally may be found in small numbers for some days after the temperature is normal In the majority of cases the cell count is normal after the first week. The gross appearance of the spinal fluid in poliomyelitis is described as clear, ground glass or hazy in appearance but rarely cloudy The usual range of the cell count is between 24 and 250 cells, but these figures do not represent rigid limits Any number of cells have been reported from 0 up to 2,000. Although the presence of a cell count over 1,000 is possible, the clinician should be especially critical in his differential diagnosis from meningitis.

The differential white cell count in the spinal fluid varies, the proportion of polymorphonuclear cells being highest at the beginning of the disease and the lymphocytes predominating later. With an individual case, however, little help can be derived from any but gross variations For instance, the finding of over 90 per cent lymphocytes in several hundred cells in the first few days of illness should put one on guard against a diagnosis of poliomyelitis There are no red cells in the spinal fluid of poliomyelitis victims except when th[illegible] the trauma of the lumbar [illegible] cells fr[illegible] since in the counting chamber red cells often appear as lymphocytes A second count should always be made of the spinal fluid drawn into a "white" pipet after the stem has been filled with glacial acetic acid to hemolyze the erythrocytes and thus prevent confusion, this technic should never be omitted and by its use spinal fluids bloody from trauma are still useful, if the white count, as in poliomyelitis, is significantly raised

TABLE 14 (*Continued*)

Year	Cases Reported		Deaths Reported		Ratio of Death Rate to Case Rate (Per Cent)
	Number	Rate per 100,000 Population	Number	Rate per 100,000 Population	
1950	33,300	22.0	1,904	1.3	5.7
1951	28,386	18.5	1,551	1.0	5.5
1952	57,879	37.2	3,145	2.0	5.4
1953	35,592	22.5	1,450	9	4.0
1954	38,476	23.9	1,368	[illegible]	3.3
1955	28,983	17.3	1,043	6	3.5
1956	15,400[b]	9.2[b]			

NOTE. Morbidity data for earlier years should be interpreted with caution because of probable underreporting of less serious cases

[a] Based on mid-year population estimates of states reporting, Bureau of the Census.

[b] Provisional data

(United States Public Health Service)

paralytic forms if, in fact, these are due to poliomyelitis virus. Probably a few deaths due to poliomyelitis are misdiagnosed, but a great many patients with nonparalytic form of this disease, or with very mild paralysis, have *not* been diagnosed at all. Therefore, the reduction in fatality rate as reported may represent an increased recognition of the milder forms of the disease, and in general it is found that in years and in areas where a high incidence is reported and where physicians are on the alert, a low fatality rate is found. Table 14 records the total incidence and death rate for the whole United States over the past 40 years. It can be seen that there is a general reduction of fatality rate with a general increase of incidence.

Prognosis in the Preparalytic Stage

At this stage we are most concerned with the probability of the appearance of paralysis. This preparalytic stage may, in fact, be a part of a nonparalytic course. Before modern techniques of tissue culture were available, of patients diagnosed as poliomyelitic in the febrile stage without paralysis from 50 to 70 per cent showed at least some slight evidence of paralysis to a skillful and careful examiner. However, probably not more than one fifth of all patients had paralysis greatly handicapping them in after life, although another fifth had a moderate paralysis which was temporary or not grossly incapacitating. However, such figures may now be challenged as more accurate viral diagnosis is available. Many patients diagnosed as nonparalytic poliomyelitis in the recent past are now being shown to have had infection with some other virus so that the per cent with paralysis will appear higher.

There is no dependable clinical or laboratory feature which will enable the physician in the preparalytic stage to make a prognosis that is anything better than an interpretation of old statistics. Although there is a tendency to believe that the higher the cell count in the spinal fluid the more severe will be the disease, this is not true. Although some statistical studies have been made which would seem to indicate a tendency the other way, there is no dependable relationship between fatality or severity of paralysis and the cell count which helps to determine the prognosis in an individual case. A fine tremor of one extremity exhibited in attempts to move it or to hold it extended sometimes precedes by a short period the paralysis of that muscle

TABLE 14 NUMBER OF POLIOMYELITIS CASES AND DEATHS REPORTED, CASE AND DEATH RATE PER 100,000 POPULATION[a] AND RATIO OF DEATH RATE TO CASE RATE, UNITED STATES, 1915-1955

YEAR	CASES REPORTED		DEATHS REPORTED		RATIO OF DEATH RATE TO CASE RATE (Per Cent)
	Number	Rate per 100,000 Population	Number	Rate per 100,000 Population	
1915	1,639	3.1	661	1.0	32.3
1916	27,363	41.4	7,179	9.4	22.7
1917	4,174	5.0	1,451	1.4	28.0
1918	2,543	2.9	1,079	1.2	41.4
1919	1,967	2.3	813	9	39.1
1920	2,338	2.4	855	9	37.5
1921	6,301	6.1	1,862	1.8	30.0
1922	2,255	2.0	847	8	40.0
1923	3,489	2.9	1,013	9	31.0
1924	5,262	4.6	1,145	1.1	23.9
1925	6,104	5.2	1,362	1.5	28.8
1926	2,750	2.2	911	8	36.4
1927	10,533	8.8	2,176	1.8	20.5
1928	5,169	4.2	1,436	1.2	28.6
1929	2,882	2.3	854	7	30.4
1930	9,220	7.5	1,427	1.2	16.0
1931	15,872	12.8	2,139	1.8	14.1
1932	3,820	3.0	882	7	23.3
1933	5,043	4.0	797	6	15.0
1934	7,510	5.9	852	7	11.9
1935	10,839	8.5	1,040	8	9.4
1936	4,523	3.5	780	[illegible]	17.1
1937	9,514	7.4	1,461	1.1	14.9
1938	1,705	1.3	487	4	30.8
1939	7,343	5.6	773	6	10.7
1940	9,804	7.4	1,026	8	10.5
1941	9,086	6.8	807	6	8.9
1942	4,167	3.1	561	4	13.5
1943	12,450	9.3	1,151	9	9.2
1944	19,029	14.3	1,361	1.0	7.2
1945	13,624	10.3	1,186	9	8.7
1946	25,698	18.4	1,845	1.3	7.2
1947	10,827	7.6	580	4	5.4
1948	27,726	19.0	1,895	1.3	6.8
1949	42,033	28.3	2,720	1.8	6.5

usually to be destroyed after one observation Intracerebral inoculation was necessary, and the subsequent disease had to be observed with autopsy confirmation usually determining the existence of the virus To determine the presence of antibodies, a mixture containing virus was first mixed with the substance suspected to contain antibodies, such as convalescent serum from a human patient or another monkey, and then injected into a monkey's brain in different proportions and doses in a crude attempt at titration. It is evident how ploddingly slow these studies had to be carried out over all these years Nevertheless, much was learned

The long-standing belief that only primates are susceptible to poliomyelitis was first shaken by the adaptation of the Lansing strain (now classified as Type II) to cotton rats and mice (Armstrong, 1939) The far-reaching effect of this discovery, which at last provided a cheap laboratory animal, is discussed in the section on Epidemiology During subsequent years further rodent adaptation was achieved with virus strains belonging to Type III (Li and Habel, 1951, Krech, 1954a), and later with Type I (Koprowski et al, 1947, Li and Schaeffer, 1953, Krech, 1954b) Infection was also produced with a Type II strain in embryonated eggs (Roca-Garcia and Jervis, 1955)

However, the important new hosts for poliovirus are now in-vitro cultures of non-nervous mammalian cells which were first described by Enders et al (1949) who produced viral multiplication in simple Maitland type cultures of several embryonic human tissues It is now known that a wide range of primate organs produce cells susceptible to poliovirus when grown in cultures The preparations most widely used are made from trypsin dispersed monkey kidney cells, either as a suspension (Salk et al, 1954) or grown out as monolayer cultures (Younger, 1956) Cell lines derived from malignant human tumors have also been employed extensively (Scherer, 1955) Recently, virus growth has been reported in chick cultures (Dunham and Ewing, 1957) and in cultures of rabbit embryo kidney (Sheffield and Churcher, 1957), but these findings need confirmation

Suffice it to say that the tissue-culture method has produced a revolutionary speed-up in the accretion of information about the biologic properties of poliovirus For example, the plaque method of tissue culture is sensitive enough to enable the identification of a single infective unit of virus and the study of its progeny (Dulbecco and Vogt, 1955) Also, the use of monkey-kidney cultures on a large scale has made possible the production of a vaccine for human use (cf Chap 24)

But tissue cultures are no longer animals, and it has not yet been possible to evaluate the importance of the changes in metabolism which induce in-vitro susceptibility to poliovirus in tissues apparently refractory in the intact animal This alteration emphasizes the need for some primate as a point for experimental reference The chimpanzee is the animal of choice for this purpose, since it shares with man sufficient susceptibility to acquire accidental poliomyelitic infection (Howe and Bodian, 1944, Howe, 1955, unpublished). Furthermore, chimpanzees are susceptible to oral inoculation by a wide range of strains of poliomyelitis virus While the paralytic rate is relatively low, virtually all of the animals without serum antibody become alimentary carriers of virus homotypic to that which they have ingested (Howe and Bodian, 1941b, Melnick and Horstmann, 1947, Howe et al, 1950, Howe, 1954, 1957) Therefore, the chimpanzee simulates man very closely in its reaction to the virus and is a most favorable animal for experiments in which this quality is of paramount importance In fact, the only recognized difference between the two species is the tendency of the chimpanzee to develop virus titers in the oropharynx higher than those of the stools, while the reverse appears to be true for man (Sabin, 1958, personal communication, Howe, 1958)

ETIOLOGY

The etiologic agent of poliomyelitis is a small virus This was established by the work of Landsteiner and Popper in 1909 when they showed that the intracerebral injection into a rhesus monkey of a bacteria-free emulsion from the spinal cord of fatal human cases caused a flaccid type paralysis characteristic of human poliomyelitis after 7 to 14 days They were able to transmit this disease from one infected monkey to others by the same

group, but fine tremors in some nonparalytic patients are exhibited quite generally in all their muscles Muscle tenderness may either conceal or simulate a paralysis. The presence of tenderness does not necessarily indicate that paralysis will occur.

The danger of occurrence of paralysis cannot be considered past as long as fever continues Usually the paralysis occurs in the 2nd or the 3rd day of the febrile period, but it may be the initial symptom to occur or it may be first detected late The likelihood of paralysis certainly becomes less as the temperature drops, although cases to the contrary are occasionally reported

Prognosis During the Acute Paralytic Stage

The prognosis for life depends upon the appearance of bulbar symptoms or paralysis of the respiratory muscles Many cases with palatal and facial paralysis occur without there being more serious complications, but difficulty in swallowing carries a more serious risk It is impossible to get accurate figures to express this in terms of percentage, since diagnostic criteria vary Certainly a large percentage of all bulbar cases will recover. A particularly high fever or rapid pulse must be considered as being especially serious Respiratory muscle paralysis varies from a barely discoverable weakness to complete loss of intercostal muscles and diaphragm. From experience with the respirator it is evident that considerable recovery usually takes place, in most instances enough eventually to permit breathing independent of a respirator, though unfortunately not necessarily with a capacity for normal life.

A dependable prognosis concerning the outcome of the paralysis found at this stage or an increase in its extent is impossible The paralysis that can be estimated during the acute stage of the disease is the worst that will occur, and some degree of recovery almost invariably takes place Occasionally, the recovery rate seems to be remarkable indeed, and extraordinary cases are encountered where extensive paralysis seems to disappear completely. Unfortunately, this is not generally true, and as a whole the worse the detectable paralysis, the worse the final crippling will be

Disturbance of motor function of the bladder and the intestine soon disappears Adequate function of the pharynx and the palate returns in a few weeks or a month in a considerable proportion of cases, though it is probable that permanent loss of function of many muscle fibers in these organs is as common as in other parts of the body. In some cases serious and permanent dysfunction results, and emotional factors play a big part Partial paralysis of the palate is easier to detect and, therefore, is more evident than that of the pharynx. The early absence of demonstrable paralysis should not be accepted as final

PATHOLOGIC PICTURE

The pathogenesis and histopathology are described in Chapter 23

EXPERIMENTAL INFECTION; HOST RANGE

It is not evident that any animal, other than man, is a natural host for the virus of poliomyelitis or is subject to disease from it The nearest approach to an exception to this statement is the chimpanzee, which in captivity has been known to acquire the disease from contact with other chimpanzees who had been infected experimentally. Furthermore, occasionally chimpanzees imported from Africa already show some neutralizing antibodies to poliomyelitis Nevertheless, it is not evident that the paralytic disease naturally occurs in these animals

The first animal to be given the disease experimentally was a rhesus monkey. Landsteiner and Popper, in 1909, were able to cause a paralytic disease in this animal by direct intracerebral injection of a bacteria-free emulsion of the cord of a human victim of the disease This experiment was the beginning of our understanding of the etiology of poliomyelitis, and for 30 subsequent years about all of our knowledge of experimental infection was derived from such costly experiments. The rarity and the high cost of these animals prevented large-scale studies and kept experiments at a level permitting scarcely more than "impressions" Each animal performed as a single expensive test tube

pH (3 8 to 8 5) for weeks but rapidly loses activity in more acid or alkaline media (Pollard and Connolly, 1949) and is very sensitive to oxidizing agents. Infectiousness diminishes very slowly in the cold, and specimens may be kept at 4° C. for months or even years if they are stored in 50 per cent glycerine-saline or some protective protein The titer of stools may be maintained without apparent diminution at −70° C for 8 years or more, despite exposure to CO_2 gas, nor is it greatly affected by repeated freezing and thawing (Howe, unpublished data) The virus also resists inactivation by alcohol, phenol, formalin, chlorine antibiotics, but, of course, is finally overwhelmed except by the last Nevertheless, several inactivating agents do not alter the virus sufficiently to destroy its antigenicity (cf Chap 24)

In view of the high prevalence of poliomyelitic infection in the summer months and in the tropics, it is surprising to find the virus rapidly rendered noninfectious by ultraviolet light (Jungblut 1937 Dick et al 1951), while its sensitivity to drying has made lyophilization impossible However, it must be admitted that these statements are based on the behavior of virus in spinal cord emulsions Virus survives in human feces for hours at summer heat and has been demonstrated in privy samples as well as in flies having access to exposed human feces (Trask et al, 1943 Trask and Paul, 1943) However, it is destroyed by routine pasteurization (62° C for 30 minutes), whether suspended in milk, cream, or ice cream (Kaplan and Melnick, 1952) but it is resistant to chlorination in proportion to the amount of organic material also present Lansing virus partially freed of cord emulsion is said to lose activity completely when exposed to 0 05 ppm of free chlorine at pH 6 85 to 7 40 for 10 minutes (Lensen et al, 1949) However, this tells little about the effectiveness of chlorination when water (especially in swimming pools) is continually subject to fresh contamination of virus mixed with pharyngeal mucus or feces In any event, the lack of evidence for the spread of poliomyelitis by swimming pools or municipal water supplies indicates that something tips the balance against the agent

Host Relationships to Infection

It is important whom one picks as his ancestors, since it is clear that selective breeding can create natural resistance to bacterial and neurotropic virus infection (Webster, 1937) Even with the random breeding habits of humans it appears that the susceptibility to paralysis is in some way hereditary This was noted some years ago by Aycock (1937), who observed that 51 per cent of poliomyelitis cases gave a history of poliomyelitis in some member of the family, while only 5 per cent of normal persons did. Obviously, it is important to pick a good control, one which resembles the group to be tested in every respect, except the quality in question. This is not easy when one is tracing family trees, but Addair and Snyder (1942) felt that they had evidence for an autosomal recessive gene in determining susceptibility to paralysis Perhaps the most interesting genetic studies of recent years are those dealing with twins One published recently by Herndon and Jennings (1951) contained a summary derived from an unselected series of 3,890 reported cases of poliomyelitis In this the authors found 45 pairs of twins, 33 dizygous and 14 monozygous, or identical. The rate for paralysis in both of the monozygous twins was 36 per cent a figure contrasted with 6 per cent in the nonidentical twins (X^2 — 6 81)

Viruses are clearly very susceptible to changes in their host and because of this they may be led in various directions For example the tissue cultures may yield so-called harmless mutants, which are not always stable and may revert to their old vicious ways when returned to the original animal host (Sabin, 1957, Koprowski et al, 1956 Dick et al, 1957)

The character of the animal host is most important for the survival of the virus, since viruses appear to be less readily adaptable to natural animal hosts than to the easy life in a tube All sorts of influences may be brought to bear on the virus by change in the host. These range from things as small as an increase in resistance to paralysis in mice receiving an inadequate diet (Foster et al, 1943), or an increased survival time in mice receiving an excess of methionine (Gershoff

technic. Within a short time the agent was identified as a virus which even in early experiments was found widely disseminated through the bodies of fatal cases, i e, in the spinal cord, the brain stem, throat washing, contents of the large intestine and occasionally in lymph nodes Although for years there was a dispute among some whether these experiments actually demonstrated a virus as the etiologic agent, in retrospect we are certain that the fact was well established at that time

In recent years this virus has been identified only in certain parts of the brain which characteristically show lesions (cf Chap 23) and in the walls of various levels of the alimentary tract (Sabin and Ward, 1941). Also, it has been found to circulate briefly in the blood during the early stages of infection (Bodian and Paffenbarger, 1954, Horstmann et al, 1954) A detailed study of orally inoculated chimpanzees (Bodian, 1956, Sabin, 1956) indicates that in this species, as in man, areas of initial growth for the virus are largely restricted to the alimentary tract with *frequent early involvement of its afferent* lymph nodes, and that there is also an inconstant and transient viremia It has been estimated that in 100 to 1,000 human alimentary infections of this general type, only one may reach the central nervous system to produce paralysis (Howe, 1949, Casey et al, 1950)

Classification of Virus

It was earlier suspected that all poliomyelitis viruses might not be the same Painstaking studies of many strains finally culminated in the knowledge that there were in fact 3 antigenically specific types These were first known by rather romantic names applied to designate a situation or person related to the isolation of the virus For instance, one of us (H.H) used the name Brunhilde which was the term applied to a noisy exhibitionist chimpanzee, the first of her species to yield virus. The name Lansing was given to a better-known strain which came from a patient in Lansing, Mich. The name of Leon was applied to a third strain from the name of a patient in California. With elaborate studies (Bodian et al, 1949, The Committee on Typing of The National Foundation for Infantile Paralysis, 1951), all strains were shown to fall into these 3 general groups and are now called simply Types I, II and III.

Physicochemical Properties of the Virus

Although the size of the poliovirus particle was originally estimated at 8 to 12 mμ by ultrafiltration of infected CNS through graded collodion membranes (Elford et al, 1935), tissue culture has made available an almost unlimited supply of virus already largely freed from extraneous proteins Utilizing this source, Sabin et al. (1954) published photographs of filtered spherical particles 25 mμ in diameter, which were thought to be the virus Schwerdt and Schaffer (1955) obtained similar results with highly purified samples of the Lansing and MEF_1 strains, utilizing methanol precipitation and differential high-speed centrifugation for the elimination of extraneous material. Furthermore, the particle associated with infectivity was found to be a nucleoprotein, as characterized by its ultraviolet-absorption spectrum It contains from 20 to 30 per cent ribose nucleic acid, which is of great interest since the Nissl bodies of nerve cells are also rich in RNA The question, as yet unanswered in this work (Schwerdt and Fogh, 1957) is the observation that the infectious unit appears to contain numerous physical particles, while Dulbecco and Vogt (1955) have indicated that a single particle is capable of initiating infection Recently, virus size has been measured by a different method, employing ionizing radiations (Henyesh et al, 1958). This technic involves determining the density of radiation required merely to abolish the demonstrable activity of the virus sample and provides data for calculating the size of each particle. This study furnishes important independent corroboration of the others

Recently, Colter et al (1957) and Alexander et al (1958) separated the RNA component of the virus particle from its protein part and succeeded in infecting host tissue with the RNA alone.

Resistance of Virus

Poliomyelitis is one of the most stable of known viruses It tolerates a wide range of

acquired (Aycock and Ingalls, 1946). There is little doubt of the effect of pregnancy itself Although many of the studies dealing with this matter are deficient in controls, this is not true for all (Anderson et al , 1952). Two small but well-reasoned studies are offered here as a model In the paper by Paffenbarger et al (1954) one is struck by the fantastically high attack rates for individuals from 25 to 34 years of age ("The parental years") These could be attributed with equal ease to increased susceptibility or to greater exposure to small children, the chief reservoir of virus In a subsequent paper (Paffenbarger and Wilson, 1955) this material was subjected to further analysis The data show a rate in pregnant contact 7 times that in a nonpregnant female group also in contact with a recognized case The poliomyelitis rate for pregnant married females was more than double that for nonpregnant married females or married males of comparable age

It had long been noted (Levinson et al , 1945, Russell, 1947, 1949, 1952, and Horstmann, 1950) that some of the most severe paralysis occurred in young people who were engaging in strenuous exercise at the onset of their illness Apparently, this is during the onset of illness and not some time before it

It seemed quite clear from their studies that the casual observations noted before could be supported by more caref[illegible] and that [illegible] ing [illegible] sys[illegible] poliomyelitis did result in a higher degree of paralytic involvement

Operative interference of any sort is also not without its dangers, although in general the public has decided to accept them There is no question of the increased risk of bulbar poliomyelitis following tonsillectomy, since virus can be found in the throats of symptomless individuals during the poliomyelitis season (Howe and Bodian, 1947) The immediate effect of tonsillectomy was first reviewed by Aycock in 1942, and it has been difficult to improve on his observations which show a concentration of bulbar cases 30 days after operation Top (1952) first noted a continued, though less striking predisposition to bulbar poliomyelitis in those who had had a tonsillectomy at any time, but it seems appropriate to return to the article of Paffenbarger and Wilson (1955), which also deals with tonsillectomy They showed that 43 per cent of the bulbar or bulbospinal cases had had a previous tonsillectomy, while this was true in only 8 to 11 per cent of other poliomyelitic infections and in 15 per cent of the control population

One need no longer worry about carious teeth as a portal of entry for poliomyelitis, despite the number of studies purporting to prove this, since in 1947 Finn, et al did a well-controlled study in which they found no difference in the prevalence of caries between 70 poliomyelitis patients and 119 siblings, who were subjected to the same conditions of exposure

A furor was caused some years ago by the discovery that injections of antigenic material, particularly pertussis and diphtheria vaccine, were prone to localize initial paralysis near the site of injection The phenomenon could be reproduced experimentally when viremia was induced artificially (Bodian, 1955),

TABLE 15 PERCENTAGE DISTRIBUTION OF THE EXPECTED PARALYTIC POLIOMYELITIS CASES * IN THE STATE OF MARYLAND BY 5-YEAR AGE BANDS, 1916-1950 †

Age	1916-1920 ‡		1946-1950 ‡	
	No Cases	Per Cent	No Cases	Per Cent
<1	133 9	12 3	51 1	4 6
1-4	743 9	68 3	320 6	28 5
5-9	144 1	13 2	319 1	28 4
10-14	35 6	3 3	135 8	12 1
15-19	12 4	1 1	87 1	7 8
20-24	13 1	1 2	58 1	5 2
25+	5 5	0 5	151 2	13 5
Total	1,088 5		1,123 0	

* Expected cases are calculated from 5 year attack rates and a standard population (1950 decennial census figures for the State of Maryland) Therefore, they are equivalent to attack rates

† Paffenbarger (unpublished)

‡ Cases for the intervening years (1921-1945) have been omitted for brevity and to emphasize the shift, during this time, from the youngest age groups (<1, 1-4 years) into strikingly older groups

et al., 1952) There are also other things which operate to change resistance to virus invasion, such as age Why is it that newborn mice are refractory to poliomyelitic virus when later on they are not (Sabin, 1950)?

There is an increased hazard of death or severe disability from "respiratory" paralysis in adults as shown in Figure 57. This was prepared from the records of carefully verified cases in Sweden, between 1925 and 1944 (Olin, 1952). The increased severity with advancing age has been confirmed independently, even to the decreased single leg paralysis and increased incidence of quadriplegia (Weinstein, 1957) The latter also noted a reversal of the preponderantly high rate among young males to females 16 to 39 years of age Since the rates for this age group are higher in both sexes than a decade ago, this probably represents increased exposure of susceptible subjects to small children, rather than the effect of pregnancy Table 15 indicates the extent to which paralytic poliomyelitis has crept insidiously into the age group of 15 to more than 25 years since 1916 to 1920. While the age specific rates are still low, the large denomination gives rise to numerous cases which contribute heavily to the undertakers and the respirator centers

There is considerable evidence that various effects in hormone balance may influence and increase the susceptibility to the paralytic consequences of poliomyelitis. Two observations in man seem to be related to this These are the vulnerability of pregnant women to severe paralysis and the increased risk of severe paralysis brought about by exercise during the invasive period of the disease. Perhaps the one most widely recognized is the increased vulnerability of pregnant women, foreshadowed by a 50 per cent incidence of still-births and abortions if poliomyelitis is

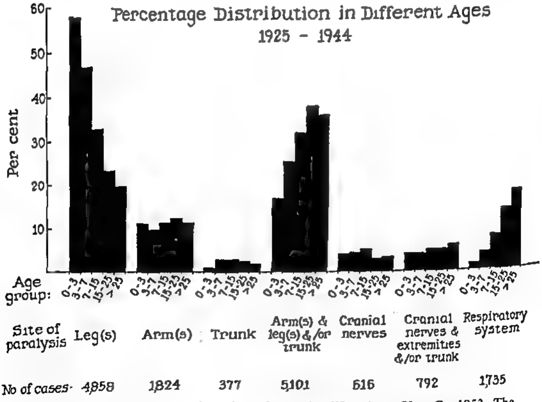

FIG. 57. Site and distribution of paralysis (Modified from Olin, G, 1952, The epidemiologic pattern of poliomyelitis in Sweden from 1905 to 1950 *in* Poliomyelitis Papers and Discussions Presented at the Second International Poliomyelitis Conference, pp 367-375, Philadelphia, Lippincott)

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It seemed quite clear from their studies that the casual observations noted before could be supported by more careful study and that indeed intense physical activity during the invasion stage of the central nervous system infection by poliomyelitis did result in a higher degree of paralytic involvement.

Operative interference of any sort is also not without its dangers, although in general the public has decided to accept them. There is no question of the increased risk of bulbar poliomyelitis following tonsillectomy, since virus can be found in the throats of symptomless individuals during the poliomyelitis season (Howe and Bodian, 1947). The immediate effect of tonsillectomy was first reviewed by Aycock in 1942, and it has been difficult to improve on his observations which show a concentration of bulbar cases 30 days after operation. Top (1952) first noted a continued, though less striking predisposition to bulbar poliomyelitis in those who had had a tonsillectomy at any time, but it seems appropriate to return to the article of Paffenbarger and Wilson (1955), which also deals with tonsillectomy. They showed that 43 per cent of the bulbar or bulbospinal cases had had a previous tonsillectomy, while this was true in only 8 to 11 per cent of other poliomyelitic infections and in 15 per cent of the control population.

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Age	1916-1920 ‡		1946-1950 ‡	
	No Cases	Per Cent	No Cases	Per Cent
<1	133.9	12.3	51.1	4.6
1-4	743.9	68.3	320.6	28.5
5-9	144.1	13.2	319.1	28.4
10-14	35.6	3.3	135.8	12.1
15-19	12.4	1.1	87.1	7.8
20-24	13.1	1.2	58.1	5.2
25+	5.5	0.5	151.2	13.5
Total	1,088.5		1,123.0	

* Expected cases are calculated from 5-year attack rates and a standard population (1950 decennial census figures for the State of Maryland). Therefore, they are equivalent to attack rates.

† Paffenbarger (unpublished).

‡ Cases for the intervening years (1921-1945) have been omitted for brevity and to emphasize the shift during this time, from the youngest age groups (<1, 1-4 years) into strikingly older groups.

thus suggesting a similar mechanism for man in whom the demonstration of this effect rested chiefly on the reversal of the usual arm-leg ratio. This means that following injections, arm paralysis became more common than leg paralysis This was noted in material from England by Hill and Knowelden (1950), in Australia by McCloskey (1950) and by Korns, et al. (1952) in the United States Others found similar results, but, excepting a most extraordinary outbreak associated with injections for yaws (Rosen and Thooris, 1953) the risk of paralysis was not great enough to interfere with immunization programs The Field Trial of the poliomyelitis vaccine was monitored very carefully in the beginning to be certain that the vaccine itself would not act as a provoking injection (Melnick and Ledinko, 1952, Francis, et al., 1957)

DIAGNOSIS

The diagnosis of the paralytic type of poliomyelitis can be made by ordinary clinical technics with a great degree of accuracy by a physician with ordinary sophistication The classic picture of fever, headache, stiff back with clear sensorium, plus the appearance of the characteristic type of paralysis is almost pathognomonic of poliomyelitis As has been said wisely, the diagnosis depends upon a shrewd clinical suspicion plus the finding of an abnormal spinal fluid compatible with the disease However, the diagnosis of acute central nervous system poliomyelitis without paralysis cannot be made firmly by simple clinical observations even with spinal fluid data This could not be stated so emphatically a few years ago, but it is clear now that other diseases, such as those due to the Coxsackie viruses and some of the ECHO viruses, can produce a clinical picture and spinal fluid changes undistinguishable from nonparalytic poliomyelitis. One should not be led into a firm diagnosis of poliomyelitis simply because of the incidence of a present epidemic, since the seasonal occurrence of these different virus infections overlap

It is important to define the circumstances in which a specific diagnosis can be made by viral and antibody studies The finding of the virus by tissue culture technics from the stools or, more important, the pharynx of patients with acute disease is highly suggestive of the diagnosis but by no means confirms it We have emphasized earlier that there is a widespread distribution of virus in people without disease particularly in epidemic times. A disease due to another virus with coincidental infection from poliomyelitis virus is quite possible. However, if the acute serum and convalescent serum obtained some weeks after the acute illness is studied for antibodies, and an increase in antibodies to the specific type virus that was found in the stool or the pharynx occurs, it is possible to establish a firm diagnosis of acute poliomyelitis. However, it is evident that confirmation of diagnosis is thus made only after the disease has run its acute course. The demonstration of a sharp rise in antibodies to a specific type of virus immediately after an acute infection with compatible symptomatology, even without the demonstration of the virus itself, is also highly suggestive

To accomplish the demonstration of the virus and of increasing antibody levels to it one must use expensive technics which cannot yet be carried out regularly in all suspicious cases and, therefore, are not readily available to most physicians As was stated, the diagnosis thus established would not be useful for the immediate care of a patient, even if the laboratory facilities were available. Therefore, during the acute stage of a disease which may be preparalytic poliomyelitis there is no way to prove that the diagnosis is not poliomyelitis and to relieve worry and tension. With the presence of the characteristic paralysis all treatment presently available can justifiably be carried out without the final confirmation from the virus laboratory.

Differential Diagnosis

The differential diagnosis needs to be considered under conditions where no paralysis exists and also under conditions where paralysis or apparent paralysis appears. In the acute preparalytic or nonparalytic stage we have to differentiate between conditions which primarily involve the central nervous system and conditions associated with extraneural disease, where we have only symptoms of central nervous involvement such as in so-called meningismus Differential diagnosis in

the acute nonparalytic or preparalytic stage can be further subdivided into the problem that faces the clinician before, and again after, the spinal fluid is available for study.

The first problem is the differentiation before a lumbar puncture is done, which often means determination of the need for doing a lumbar puncture. It is clear that symptoms like those of acute preparalytic poliomyelitis can be associated with many non-neurologic diseases. A mere listing of these diseases would accomplish little, and there is not space to discuss each one separately. Common respiratory tract infections, particularly influenza, can be particularly puzzling since in both there are general aches and pains, muscle soreness and an alert sensorium. Headache, fever, vomiting and a stiffness of the neck or the back may be detected by the physician who is thinking of the possibility of poliomyelitis. In a somewhat similar manner rheumatic fever can be confusing, since we find headache, pains in the joints which, especially in children, may be interpreted as muscle pains and, when the spine is involved, a stiff neck and back makes a picture strikingly similar to poliomyelitis. As is well known, the onset of many acute febrile illnesses in children is associated with headache and stiff neck and back in a syndrome commonly called meningismus.

If the symptoms lead the physician to decide that a lumbar puncture is indicated, and the spinal fluid shows an increase of cells, the conditions mentioned are promptly excluded, and only diseases with actual involvement of the central nervous system have to be considered. First, in order of importance since specific therapy is available, is purulent meningitis, then parameningeal septic infections and finally other virus diseases of the central nervous system. It is very important to evaluate the sensorium. It is true that in a small percentage of poliomyelitis patients the sensorium is dulled, and there is confusion and apathy. Almost never, however, are there convulsions and seldom deep coma. In purulent meningitis the sensorium is often disturbed markedly, and patients are stuporous if not unconscious, and they frequently suffer convulsions. Ordinarily, therefore, the differentiation is easy even without aid of spinal fluid examination, but much help is obtained from spinal fluid examination in most cases. Mumps meningitis with parotitis and lymphocytic choriomeningitis can give a picture quite similar to that of nonparalytic poliomyelitis, but the usual spinal fluid in mumps with nearly 100 per cent lymphocytes in a total count of several 100's early in the acute disease makes the differentiation easy. Brain abscess or extradural abscesses can cause fever, headache, vomiting, stiff neck and back and spinal fluid changes which may simulate those of poliomyelitis. The more septic nature of the disease and the presence of an otitis or sinus infection greatly aids the differentiation. Purulent meningitis itself usually has a cell count in the thousands with low sugar and the presence of micro-organisms. If from spinal fluid examination we have ruled out mumps and purulent meningitis, we have a variety of other diseases to consider, among which occur with increasing interest the Coxsackie group and some of the so-called ECHO group. It was stated above that ordinarily by clinical or spinal fluid examination no exact differentiation can be made between nonparalytic poliomyelitis and Coxsackie or ECHO virus infections. There are other conditions which cause encephalitis where the degree of involvement of the encephalon or the presence of convulsions can rather certainly rule out poliomyelitis. Often, however, in a patient without coma, convulsion or paralysis, the clinician is unable to make a certain diagnosis of poliomyelitis until eventually he is aided by data from the virus laboratory.

Differential Diagnosis in Disease with Signs of Paralysis. Where paralysis or the resemblance of it is apparent, this symptom becomes the leading point in the diagnostic attack. Paralysis in poliomyelitis may be encountered first in the acute febrile meningeal stage or as an isolated symptom after the febrile period is past. The approach to the differentiation should be made by orderly steps: (1) determination of the actual existence of paralysis instead of a "pseudoparalysis" due to pain, (2) differentiation between upper and lower motor neuron disease, (3) if it is determined that an actual flaccid, lower motor neuron paralysis exists, differentiation from diseases functionally disturbing other parts of the cord as well as the motor

cells; and (4) if only motor lesions occur differentiation from several types of so-called "peripheral neuritis."

Differential diagnosis of the bulbar and the respiratory paralyses need to be considered separately from paralyses of the trunk and the extremities.

Pseudoparalysis of Trunk and Extremities. One group of diseases confused with poliomyelitis, especially in children, is that which produces tenderness of extremities, so that a patient's unwillingness to contract a muscle because of pain may be interpreted as paralysis

Tenderness and a pseudoparalysis which simulates poliomyelitis to the poliomyelitis-conscious physician or parent can be caused by a host of conditions in young children following unreported trauma Scurvy in nonepidemic and epidemic times has been confused with poliomyelitis Syphilis can cause a similar picture in small infants The appearance of paralysis occurs sometimes in Sydenham's chorea, and one extremity may appear limp and flaccid A pinprick will usually make it evident that muscle power is present

Differentiation of Actual Paralysis of the Trunk and the Extremities. Exclusion of Upper Motor Neuron Conditions. Having determined that a paralysis is real and not simulated, the next step should be to determine whether it is spastic or flaccid, that is, whether the lesion is in an upper motor neuron or due to damage to the lower motor neuron Ordinarily, differentiation is easy, but quite commonly the appearance of an upper motor neuron lesion is, for a few weeks or longer in small children, that of a flaccid paralysis, and it is quite difficult to determine the basic mechanism Evidence of increased intracranial pressure, of increased reflexes in other extremities or of eyeground changes often makes a differentiation clear A history of a convulsion preceding the flaccid stage of an upper motor neuron lesion is quite common, and convulsions must be exceedingly rare in poliomyelitis

Exclusion of Other Than Motor Cell Lesions in the Cord. Faced with a flaccid paralysis, one should determine first whether or not there are true and dependable changes in sensation If there is clearly a loss of sensation to touch, to pain, or to position sense, poliomyelitis can be ruled out quickly. The picture of a transection of the cord or transverse myelitis itself is usually quickly distinguished from poliomyelitis by the most gross form of neurologic examination.

Exclusion of Peripheral Nerve Lesions. When all of a nerve trunk is so involved as to prevent its functioning, evidence of sensory nerve distributions may rule out poliomyelitis fairly quickly. When only the motor part of a single nerve trunk is involved, knowledge of the distribution of a nerve is helpful in differential diagnosis It is characteristic for a peripheral nerve lesion that extensively involves an arm or a leg to bring about a paralysis of the fingers and the toes as well as of the great muscles Poliomyelitis, on the other hand, may cause a complete paralysis of the whole extremity except the toes or the fingers

A number of diseases occur which cause lower motor neuron palsies, but all of these conditions cannot be discussed separately. The nomenclature is confused, but multiple neuritis, peripheral neuritis or postinfectious neuritis or neuronitis, and the Guillain-Barré syndrome are favorite terms The coincidence or history of immediately preceding infection with the virus of measles, chickenpox or vaccinia often makes the etiologic agent clear. In general, as distinct from poliomyelitis, the paralysis of these diseases is more diffuse in appearance, obviously progresses over a period of days, and, unless death occurs, final recovery is much more likely to be complete. Lead-poisoning and postdiphtheritic paralysis especially should be considered.

Bulbar or Respiratory Muscle Paralysis. The symptoms produced by paralysis may be misinterpreted, and the existence of paralysis be unrecognized This is particularly likely when respiratory distress or difficulty in swallowing is the presenting symptom of the disease as first seen by the physician

Pneumonia. Although it would seem to differ greatly from poliomyelitis, pneumonia can be confused with respiratory muscle paralysis, when meningismus is present and there is a striking difference in excursion of one side of the thorax The opposite mistake may also be made.

Difficulty in Swallowing. Confusion in diagnosis is quite common when the present-

ing symptom offered by a patient is that of inability to swallow The victims of infantile paralysis, when young, may not at first understand their own difficulty When medical aid is sought belatedly or the disease is rapid in course, the patient may present himself first in a state of coma or semicoma with noisy, interrupted breathing and with obvious and marked respiratory difficulty The condition has been confused with *overwhelming pneumonia* or *sepsis*, the noisy, stertorous breathing suggesting that often seen in moribund patients A key to the proper diagnosis should be apparent, particularly if any consciousness on the patient's part can be detected Usually the patients themselves, unless indeed semiconscious and near death, will show great apprehension or terror When the patient is old enough to recognize his difficulty early in the course of his trouble, or when it is evident from watching a patient try to drink some water that there is inability to swallow, a *foreign body causing obstruction to the esophagus* is apt to be considered, and more than one child with pharyngeal paralysis unfortunately has been subjected to esophagoscopy.

Diphtheria has to be considered when palatal paralysis is recognized The presenting symptom is nasal speech and nasal regurgitation on attempts to swallow The absence of diphtheritic membrane in the throat, of course, does not rule out this condition The knee-kicks in diphtheritic paralysis are lost early, while in the acute stage of poliomyelitis, during which palatal paralysis is encountered, the knee-kicks may be exaggerated, if changed at all from normal In diphtheria, ocular palsy is quite commonly associated with palatal and pharyngeal palsies, while it is rare in poliomyelitis

A *cerebral embolus*, a *brain tumor*, and even a *retropharyngeal abscess* can cause symptoms that can be confused with bulbar poliomyelitis

Encephalitic Type of Poliomyelitis While ordinarily the patient with poliomyelitis is alert, at least when aroused, and quite conscious of what is going on, occasionally we see a patient excessively dulled and stuporous, lying in coma The encephalitic sensorium quite commonly is associated with the bulbar type of paralysis but may occur as well in cases with spinal paralysis or no paralysis at all Here the differential diagnosis lies between poliomyelitis, tuberculous meningitis and all the diseases classified under the term encephalitis

Differential Diagnostic Value of Spinal Fluid Findings. A great number of diseases produce changes in the spinal fluid similar enough to those of infantile paralysis to cause confusion

PURULENT MENINGITIS The spinal fluid of poliomyelitis shows a relatively low cell count Any cell count over 1,000 or any cloudy fluid is due more likely to a purulent meningitis than to poliomyelitis In purulent meningitis the spinal fluid sugar usually is reduced, while in poliomyelitis it is increased or normal Cultures and smears for demonstration of micro-organisms always must be made carefully, whatever the presumptive diagnosis.

TUBERCULOUS MENINGITIS The spinal fluids of poliomyelitis and tuberculous meningitis have the same characteristics except for the sugar content, which in tuberculous meningitis is significantly reduced.

PARAMENINGEAL INFECTION A collection of pus near the meninges, either a brain abscess or an extradural abscess such as those associated with mastoid infections, can produce a spinal fluid identical with that occuring in poliomyelitis

Other Virus Infections of the Central Nervous System. From a study of the characteristics of spinal fluid in the clinical laboratory, no features can be detected which enable one to distinguish with certainty between poliomyelitis and a long list of other virus diseases which can affect the central nervous system, such as measles, chickenpox, vaccinia encephalitis, equine encephalitis, St [illegible] encephalitis will produce a characteristic spinal fluid change, when the total count is high, with such a high percentage of lymphocytes that one would consider it incompatible with poliomyelitis

SYPHILIS There are no characteristics of the spinal fluid, excepting the serologic tests (Wassermann, Kahn, etc.), which distinguish syphilis from poliomyelitis

MULTIPLE PERIPHERAL NEURITIS OR NEURONITIS In most cases of toxic neuronitis, Guillain-Barré syndrome, or infectious polyneuritis, only an increased globulin value is found, although rarely a small increase of white cells occurs In lead-poisoning counts as high as 100 or 200 cells are sometimes found Of course, similar spinal fluid findings occur after the acute febrile course of paralytic poliomyelitis.

Poliomyelitis With a Normal Spinal Fluid Can acute poliomyelitis occur with no abnormalities in the spinal fluid? There certainly can be infection with the virus of poliomyelitis without involvement of the central nervous system, but can the disease attack the central nervous system violently enough to produce paralysis and still produce no changes in the spinal fluid? This must be possible However, there often can be a simple explanation for the findings of a negative spinal fluid in the acute stage of the clinically recognizable disease A negative spinal fluid can be obtained if lumbar puncture is done too early, within a few hours of the onset of meningeal symptoms, and also too late, as in some instances the cells apparently disappear very early.

All cases cannot be so explained, nevertheless, we must conclude that it is possible, though rare, to find a normal spinal fluid during the acute state of poliomyelitis in a patient with evidence of paralysis However, great caution must be exercised in making a diagnosis of poliomyelitis under these circumstances

TREATMENT

There is no specific treatment for the disease Stated differently, we can say that no form of therapy now known seems to influence the course of action of the virus on the central nervous system once the virus has entered nerve cells Although the history of poliomyelitis is filled with therapeutic adventures toward direct therapy, there is no good evidence that actual damage to anterior horn cells, which is the specific lesion that makes this disease important, is prevented by any procedure now known A short review of some of the better-known therapeutic efforts in the past seems to be justified.

TREATMENT WITH HUMAN "IMMUNE" SERUM

The demonstration of poliomyelitis antibodies in the blood of human convalescents was recognized early as offering a possible mode of treatment. Accordingly, extensive experiments were carried out with pooled convalescent serum and human adult serum given to patients in the early preparalytic stage of poliomyelitis Hundreds of patients were so treated, and the results furnished a classic example of the hazard of clinical studies of poliomyelitis without proper controls. It was first found that a great deal less paralysis occurred in patients treated with convalescent serum in the preparalytic stage of poliomyelitis than was found in patients who were not so treated but were diagnosed first because of paralysis (Aycock et al, *1929*, Harmon, *1934*). When finally studies were made treating only alternate patients diagnosed in the preparalytic stage, no significant difference in the degree of paralysis that later developed was observed (Kramer et al, *1932*; Park, *1932*) Later studies with gamma globulin (Bahlke and Perkins, *1945*) have also shown no beneficial results in therapy after central nervous system involvement.

TREATMENT BY PROCEDURES DIRECTED TOWARD THE CIRCULATION IN THE CORD

As discussed in Chapter 23, a striking feature in this disease is the perivascular infiltration of the cord with lymphocytes From this it was argued that the damage to the nerve cells themselves was due to anoxia or some other effect resulting from reducing the circulation to the anterior horn cells. Accordingly, therapeutic efforts were directed toward preventing or dispersing the collection of cells around blood vessels of the cord and toward reducing the edema of the brain and the cord Repeated or continuous drainage of spinal fluid was recommended Hypotonic solutions were administered parenterally, and large amounts of water were given by mouth while continuous spinal drainage was maintained Hypertonic fluids were administered intravenously to reduce the edema Adrenalin was given intraspinally All these methods had eloquent advocates, with striking results re-

ported in very small and uncontrolled groups of patients Treatment with both hypotonic and hypertonic solutions has received equally enthusiastic support Since no well-controlled studies of any of these methods of therapy have been presented, we are forced to look on all these efforts as rather desperate attempts to "do something" The concept, so clearly developed by Bodian, that the virus first attacks the great motor cells and that the edema and perivascular infiltration are only secondary, destroys the logic of such treatments It is still quite possible, however, that the secondary interstitial changes may exaggerate the damage produced by the virus, and attempts at a more critical evaluation of such methods of therapy still may be justifiable

Concepts as to the nature of paralysis and its treatment that were considerably different from what had been customarily accepted in the past were advocated by Elizabeth Kenny of Australia and her disciples (Pohl and Kenny, 1943, Pohl, 1945) Greater emphasis was placed on the heat treatment of tender muscles during the acute stage, not simply to relieve pain but as a procedure actually preventing paralysis Immobilization by casts and splints was discarded Muscle re-education with active participation by the patient was begun early in the disease as soon as muscle tenderness, relieved by the hot packs allowed it

Miss Kenny and her followers made broad claims for the efficacy of such treatment These claims reached the public, and her method of treatment was carried out widely There was much that was good in the treatment, but the explanatory theories were poorly founded They taught that there was actually no true paralysis, as it is ordinarily understood in poliomyelitis, but that all paralysis resulted only if tenderness and spasm were not reduced or eliminated by the use of hot packs applied in a very specific manner Their explanation of the physiologic disturbances in poliomyelitis hardly deserves more discussion However, the effectiveness of the Kenny school in muscle re-education almost balances in good the lack of sound theory and the devotion to certain unimportant details

Unfortunately, as has been true with other adventures in therapy, attempts at treatment of the paralysis of poliomyelitis have been carried out without proper control studies, without proper muscle examination, and apparently with a great lack of experience and knowledge as to what happens in the natural course of the untreated disease Since, even with the most meticulously careful muscle examinations, a good number of all patients [illegible]

can be seen how easy it is to give credit to any form of therapy that is generally applied without carefully planned controls during the acute stage of the disease Much of the apparent paralysis is transitory and, moreover, when pain or spasm exists, it is extremely difficult to differentiate true paralysis from muscle inhibition due to pain It has long been noted that improvement in muscle function takes place over periods of months and years It is hard to determine how much of this has taken place by nature alone and how much has been effected by treatment of some sort that has been carried out, but a certain amount of improvement always takes place no matter what the treatment Sudden, almost dramatic, improvement has occurred and has been reported at various stages, sometimes after periods of months, both with no treatment and after some particularly well-advocated treatment Obviously, no form of therapy in this disease can be accepted as effective unless one critically evaluates it in the light of the spontaneous variations which may take place

General Therapeutic Measures

Rest. Excellent studies strongly suggest that the value of rest in the preparalytic and the early paralytic state of poliomyelitis may be more specific and considerably greater than we are apt to consider it in other diseases, and that activity or rest may actually make the difference between permanent destruction and temporary incapacity of certain nerve cells The evidence supporting this idea is derived from the observation that many patients with the worst paralysis gave a story of continuous activity, sometimes violent, during the very early hours or days

of the disease. It is not apparent that exercise during the incubation period or the "dromedary" period is of importance The ill effects of such activity have been supported by several good studies (see above). It seems justified to make every effort to insist that absolute rest be carried out at the first sign of illness due to poliomyelitis or, in epidemics, at the first symptom of any undiagnosed febrile illness. This is good theory but often hard to put into practice without frightening the public

Fluids. Fear is often expressed of giving too much fluid, particularly parenterally, to patients suffering from edema of the medulla. The tendency of inflammed tissues to give up fluid less readily and take it up more readily seems to be established, and a reasonable program should make a compromise between overhydration and dehydration, therefore, a moderate amount of fluid with consideration for the patient's fever and sweating and, most of all, his comfort, should be allowed

Care of the Skin. These patients sweat profusely and are partially immobilized both by tenderness and actual paralysis Care of the skin is further complicated by the application of hot, wet packs to tender muscle groups. Especially careful nursing attention must be given

Comfort. Patients with poliomyelitis may suffer much pain The treatment of muscle tenderness with heat is discussed below The physician must be particularly concerned in directing the nursing of infants and small children, who may be handled by their attendants without any proper understanding of the degree of pain that is caused Attempts to evaluate paralysis by the doctor, changing of diapers and other routine procedures by the nurse should be carried out with the greatest gentleness

Intestinal Stasis. Disturbance in the function of the intestine and paralysis of the bladder need particular attention during the acute stage of the disease. Constipation is marked in many patients and probably is at least partially due to a disturbance of peristalsis brought about by the disease Intestinal stasis is temporary and lasts only a few days, but abdominal pain due to the constipation should be relieved by small enemas gently administered.

Urinary Retention. Bladder paralysis also seems to be temporary, but few careful follow-up studies have been made. Relief may be obtained by catheterization after it has been demonstrated that the patient cannot void spontaneously. The risk of catheterization is justified by the marked urinary retention and the pain which otherwise would occur Usually 2 or 3 catheterizations will be enough to carry the patient over this phase of his disease.

Treatment of Paralysis

There is no specific treatment for the paralysis, but many nonspecific steps may make important differences in the final outcome The treatment of paralysis caused by poliomyelitis will not be discussed in this chapter at any great length. The complex details involved in immobilization of paralyzed muscles, different types of physiotherapy, muscle training and surgery with its various corrective and stabilizing operations are too specialized for proper consideration here. Certain simple principles of treatment may be outlined, although at the risk that oversimplification may lead to inaccuracies. Most orthopedists agree on the following general procedures:

1 Protection of paralyzed muscles to be effected by suitable splints, casts or other means of fixation for the period of muscle tenderness or spasm which may last several weeks. This should not be carried to extremes, and motion up to the point of pain only should be permitted.

2. The application of heat during the acute stage to tender muscles by the use of hot packs. This has been emphasized always as important for comfort, if not for any specific effect on the course of the disease that might result

3 The cautious initiation of physiotherapy technics for the stimulation and the re-education of weakened muscles and prevention of contractures, always carried out to a point short of fatigue, with immobilization being reapplied in the hours of the day not given over to such physiotherapy.

4 During a final stage, after most muscle function has returned that will return, the use of muscle transplant and stabilization operations on the joints to permit the most efficient function of residual muscle power.

The relief of pain in the acute stage by the use of hot packs applied sensibly in moderation over a limited number of days or weeks and on the basis of definite subjective relief is generally accepted. There has been a great reduction in the use of immobilization technics, and opportunity is given the patient to move around in bed and to use partially paralyzed muscles to the limit of his desire, short of fatigue and pain Gradual attempts at passive motion and muscle re-education are accepted as of very great importance The technic of muscle re-education requires specially skilled technical workers with a good knowledge of functional anatomy and a personality that can induce a timid patient consciously to endeavor to use each muscle Undoubtedly, great improvement in function takes place as a result of skillful muscle reeducation, not only in poliomyelitis but also in many other conditions whether or not associated with pain Ultimately, the use of supporting braces, splints or stabilizing operations to allow the patient to make whatever use he can of what muscles he has left is of great importance.

Treatment of Paralysis Leading to Respiratory Failure

The manner in which poliomyelitis may lead to respiratory failure has been described above The successful treatment of respiratory difficulty in poliomyelitis depends upon an exact clinical analysis of the functional defect Although we can no more prevent the poliomyelitis virus from causing bulbar or respiratory paralyses than we can prevent it from causing paralyses of arms or legs, nevertheless, even symptomatic treatment may make the difference between life and death in the forms of poliomyelitis that may lead to respiratory failure Even so, too often at autopsy the extensive destruction of the brain stem with its "vital" centers demonstrates the futility of any form of treatment now known.

Although elaborate programs have been outlined for the treatment of the complex conditions leading to respiratory failure, only the relatively simple procedures are surely effective Furthermore, although there are frequently multiple palsies in the individual patient, the different effects must be untangled and a logical program directed toward the difficulties as they present themselves Therefore, we must consider separately respiratory distress from respiratory muscle paralysis, from pharyngeal paralysis, from "central" disturbance of respiratory control, and their combinations

Signs and Symptoms. The early symptoms of respiratory involvement result from increased respiratory effort rather than from actual respiratory failure Many attempts have been made to use elaborate laboratory methods for the early detection of respiratory insufficiency, specifically, the determination of oxygen saturation levels of hemoglobin and of blood carbon dioxide levels However, in spite of respiratory muscle weakness, increased effort in breathing may be successful in maintaining normal or even increased alveolar ventilation Therefore, neither of these determinations nor any other blood chemical measurement now available is likely to prove useful in detecting early respiratory weakness and, in fact, may be misleading (Dickinson et al, 1953) Determination of the reduction of vital capacity, especially in adults, is helpful, but in the absence of predetermined levels of the patient's normal capacity, early and moderate reductions may not be detectable

Many patients with acute poliomyelitis, either with or without moderate degrees of respiratory muscle weakness, succeed in breathing with such effort that actually overventilation of the lungs, leading to alkalosis, results This probably is due to anxiety and fear Hyperventilation, in our experience, is more characteristic of early respiratory muscle weakness than is actual failure of respiratory effort (Dickinson et al , 1953)

In order of likely sequence, we regard the following as clinical signs first, of increased respiratory effort, and then of respiratory insufficiency

Increased Respiratory Effort

1 Increased rate of breathing
2 Dilatation of nostrils
3 Interruption of speech
4 Use of accessory muscles of respiration

Effort and/or Failure

5 Anxiety
6 Restlessness or sleeplessness
7. Disorientation

Respiratory Failure

8 Coma
9 Cyanosis
10 Convulsions

Anxiety, restlessness and disorientation may be evidence not only of effort or fatigue but also of actual failure of respiratory function, that is, of hypoxia or carbon dioxide retention Coma, cyanosis and convulsions can surely indicate respiratory insufficiency, easily shown by carbon dioxide determinations or oxygen saturation levels of hemoglobin but usually long after the clinical signs of dyspnea are clearly evident Furthermore, disorientation may be the result of fatigue alone as well as of carbon dioxide retention or oxygen lack However, such fatigue may lead soon to respiratory inadequacy Cyanosis is likely to be an agonal symptom unless for short periods due to remediable airway obstruction

A valuable early sign of respiratory effort and reduction of vital capacity is interrupted, staccato speech It is quite characteristic to hear a patient speak with only several syllables to each breath.

Demonstration of a moderate degree of paralysis of the diaphragm or of the intercostal muscles can be aided if dyspnea is caused or made greater by splinting the chest or the abdomen by one's hands.

Respiratory Muscle Paralysis One could consider the use of the respirator for respiratory paralysis as desirable not only to save life in extensively paralyzed patients who would otherwise die but also to give rest to those partially paralyzed patients with only slight dyspnea The relief to the latter patients is often far more striking than would be expected Not infrequently, symptoms of mental confusion due to anoxia or accumulative fatigue may be relieved when the patient is given respirator treatment, in spite of the fact that it was not obvious that the muscles of respiration were severely paralyzed.

An original justification for the use of the respirator in severely stricken patients rested wholly upon the possibility that eventually the respiratory muscles would improve If improvement could not take place to an extent allowing the patient eventually to become independent of such a device, and if the respirator treatment only led to a persistence of this tragic situation, its use could hardly have been justified. However, experience has taught that no absolutely bad prognosis for recovery can be so certainly made that withholding the use of the respirator at the time when its use is first indicated can be justified. Some degree of recovery almost always occurs, and occasionally very remarkable return of function is evident. It is logical to use such aids to respiration in early respiratory muscle paralysis whenever they provide rest and comfort and are not themselves a cause of persistent anxiety and fear.

Luckily, even in badly paralyzed patients, control of alveolar ventilation is not wholly lost, so that a reasonably efficient regulation of respiration can be maintained by rough adjustments of pressure and rate possible in these machines.

Besides the standard tank respirator, the cuirass type respirator and the Rocking Bed, and the various positive pressure devices are useful None is so efficient and dependable that it can well be substituted for the tank respirator early in the course of extensive respiratory muscle paralysis, but these devices are of great aid in weaning patients from the tank respirator.

The time for initiating respirator care cannot be stated arbitrarily because the patient's emotion influences the decision If, in spite of proper psychologic preparation, he is greatly frightened by this coffinlike machine or any other mechanical equipment, especially applied over the mouth, he may prevent its early effective use, even if it does give him some rest. Often only a trial of the machine will determine whether or not it will be effective.

Respirator Addiction. One of the most puzzling problems in the care of patients in a respirator, particularly with emotionally labile people, and probably more with adults than with children, is addiction to the respirator or overdependence on it. As mentioned above, hyperpnea, alveolar overventilation, is also seen before any mechanical device for the aid of respiration has been used, and this phenomenon seems to be due to fear Therefore, the word "addiction" may not always be appropriate, since the habit of overventilation was established before dependence upon

artificial respiration could have developed However, persistent hyperventilation establishes an abnormal pH and arterial pCO_2 Renal compensation may then return the pH to normal, after which continued hyperventilation becomes necessary to maintain a normal pH This might be considered as physiologic addiction.

Adjustment of the Respirator We cannot hope here to detail directions for running a tank respirator for the care of a patient with respiratory muscle paralysis Let us say, however, that the task is not as difficult as it might first seem Usually a rhythmical negative pressure of 15 cm of water at a rate of 16 to 20 a minute is adequate for normal gas exchange. When the respirator was invented it was with considerable trepidation that it was first used in fear that with such a crude instrument the marvelously delicate maintenance of balanced pulmonary ventilation that the body regulates never could be approached and that we would be constantly in the position of producing acidosis or alkalosis However, it became clear that few patients are fully paralyzed and that most can control their respiration to a certain extent The closing of the glottis can prevent overventilation to a certain degree if the patient chooses to do so, and usually he can aid inspiration We have already emphasized the psychic disturbance which leads to overventilation, and the physiologic disturbance which continues a tendency toward overventilation However one can hardly have a constant blood oxygen or alveolar pCO_2 analysis recorded on the patient Even if these data were available, it might not be a good guide as was explained earlier A good clinician can learn much by simply watching his patient, particularly when the patient is asleep or unaware that his respiration is being studied A patient with inadequate pulmonary ventilation, or at least one not satisfying his requirements, whether or not they are normal requirements, will show some dilatation in the alae nasae or he may show use of his accessory respiratory muscles in the neck or may take extra breaths between those the machine initiates This should be an indication to step up the rate or the pressure of the machine If the patient is overventilated and has not accepted it, he may close his glottis, causing a noise of inspiratory obstruction, or one may notice a contraction of the abdominal muscles during the inspiratory phase of the machine, which is very revealing if one looks for it. These clinical guides are sound and should not be neglected in this age of blood chemistry

Pharyngeal Paralysis In treating any patient with pharyngeal paralysis, aspiration of the secretions from the throat frequently is of lifesaving value, and suitable apparatus for accomplishing this always should be at hand Careful attention should be given, when motor-driven suction is available, that too great suction does not traumatize the mucous membranes As little aspiration should be carried out as is necessary to keep the patient comfortable, as in certain excitable patients the procedure may irritate the pharynx, increase the apprehension of the patient and so increase the production of mucus In some patients such great relief is afforded by aspiration that they request it and sometimes can carry it out themselves In children much tact and reassurance, particularly at initiation of treatment, is necessary A metal aspirator tube that can be placed in the throat exactly where it is needed is most effective A rubber tube, although it seems soft and least likely to be traumatic, often cannot be directed effectively and will increase the gag reflex if it touches the postpharyngeal wall

Atropine, used to dry up secretion, does more harm than good It may result in the production of thick, sticky secretions which are even more difficult to remove, and the effect of atropine itself on the rate of the heart in bulbar poliomyelitis should be avoided

The handling of the excitable and nervous patient needs special emphasis Anxiety and fear of choking make patients with pharyngeal paralysis much more difficult to treat The more frightened they become the more the secretions bother them and the greater their fatigue becomes so that aspiration of the throat can be carried out only with such a struggle as greatly to diminish its value A calm, reassuring attitude, deliberate and firm action on the part of the doctor and the nurse, and the avoidance of a state of confusing bustle and hurry are of crucial importance.

In spite of the greatest skill by nurses and physicians in preventing aspiration into the lungs and in keeping the pharynx free of secre-

tions, success will not always follow. Although in many cases of pharyngeal paralysis it can be avoided, a tracheotomy to produce a free airway and allow sleep has been advocated for many years in certain patients (Wilson, 1932), particularly in the high-strung, nervous type. Since this time, especially in the last 8 years, much more experience and enthusiasm in the use of tracheotomy has been reported (Glaser, 1945, Brown and Baker, 1949). One of the best studies was by Plum and Dunning (1956). An acute attack of cyanosis due to choking on aspirated secretions or vomitus seems seriously to aggravate the underlying pathologic condition, at least, after such an attack patients are often dramatically and permanently worse. It seems quite possible that generalized hypoxia has a direct effect on the course of the disease in the central nervous system The use of tracheotomy to prevent such a situation is therefore clearly indicated The problem is to carry out the tracheotomy before an acute attack of cyanosis makes it obvious to everyone that it should have been done earlier and yet not make the procedure so routine that many unnecessary tracheotomies are done It requires good judgment and careful observation to determine this point A tracheotomy is a serious and mutilating operation and is a step not to be undertaken lightly Unfortunately, tracheotomy has been advocated with such enthusiasm that many practitioners believe ill-advisedly that it is indicated for all cases of "bulbar" poliomyelitis, or worse, for all respiratory distress

The need for this operation usually can be determined by a few hours of critical observation The emotional attitude of the patient, his response to aspiration, postural drainage and oxygen therapy can give one a good idea of the need for tracheotomy before one gets into great difficulties One should demand that a patient under a conservative method of care should be able to get such relief that he can relax and go to sleep If he cannot relax, if sleep is constantly interrupted, the condition will get progressively worse, since fatigue will greatly prejudice the chances of a successful outcome, and tracheotomy is clearly indicated.

Sometimes it is extremely difficult to determine whether the irregular respirations are due to pharyngeal secretions or to damage to the respiratory centers themselves in the brain stem. If the respiratory disturbance is central in origin, a tracheotomy does not seem to be logical unless artificial respiration is indicated because the patient's own efforts are ineffective. However, it is often difficult to make a clear distinction as to which is causing the symptoms and, if uncertainty exists after careful observation, the operation is justified.

In the last 4 or 5 years the use of positive pressure breathing devices, attached to a tracheotomy tube, have been increasingly valuable. The widespread use of this in Europe followed from the studies of Scandinavians (Engstrom, 1954). A tracheotomy tube with a cuff which made an airtight connection in the trachea was generally used, so that full control of respiration was given over to the machine. Although much has been learned by the development of these devices, one could question whether their use was as wise as was first thought. Tracheotomy apparently was used very indiscriminately for all patients with respiratory distress. In this country more conservative use of positive pressure devices with a tracheotomy has developed and undoubtedly has been lifesaving Positive pressure respiration, without an airtight tracheal connection, has become extremely useful (Morch et al, 1956). This is particularly true in patients who have to have tracheotomy anyway and in whom the tracheotomy is not indicated simply to make it convenient to give positive pressure respiration when other devices could be used Cumbersome as it is, the use of the tank respirator seems to be far wiser except where a tracheotomy is indicated for pharyngeal paralysis or loss of central control The disadvantage of a tracheotomy with an airtight cuff to the trachea is that the constant pressure leads to ulceration and hemorrhage in some cases, though the first advocates of this technic themselves do not look on this risk as serious. Although undoubtedly the greater use of tracheotomy has saved lives and is amply justified, the great disadvantage is the trouble in eliminating it once it has been used, and particularly after use for some time. There is not only the psychic problem in getting rid of the tracheotomy too, which is reminiscent of the

problem of getting the patient out of the respirator, but there is a real pathologic problem in the risk of inflammatory processes in the larynx which causes laryngeal obstruction as well as the chronic inflammatory condition in the trachea itself The mechanical disadvantages of taking care of a patient with a tracheotomy who must also be in a tank respirator are great, though the problem has been managed successfully many times The care of a patient outside of a tank is obviously much simplified

Laryngeal Paralysis. Laryngeal paralysis can be quickly lethal if it involves the abductor muscles bilaterally by obstructing the airway through the larynx Warning signs are hoarseness or aphonia. Tracheotomy is the only effective treatment In an emergency, the insertion of an intratracheal tube through the pharynx may be life-saving until a tracheotomy can be performed

Involvement of the Respiratory Centers. When the respiratory centers are involved, death from respiratory failure can occur with a patent airway and normal respiratory muscles, because the co-ordinated drive for breathing has been lost Such patients breathe very irregularly, they characteristically take a deep breath followed by several ineffectual breaths and may have frequent periods of apnea They are more apt to breathe effectively though irregularly when stimulated, only to become cyanotic and apneic when left alone or when they fall asleep These patients, in contrast with those with respiratory muscle paralysis or airway obstruction, can develop actual respiratory failure and die with no signs whatever of dyspnea. They are usually completely unable to synchronize with a mechanical respirator, although such treatment may be lifesaving because the machine converts an occasional small ineffectual breath into an effective one.

Several years ago the electrophrenic respirator was devised for this type of patient (Sarnoff et al, 1948, 1950) By completely blocking the inefficient, irregular efferent impulses from the malfunctioning respiratory center and by providing artificial rhythmic stimuli, this device produces completely controllable respirations However, it requires a normal phrenic nerve In our experience it is the rare patient indeed who is helped by its use.

Recently, a number of physicians have suggested that the patient with a malfunctioning respiratory center who is unable to synchronize with a mechanical respirator may be saved by administering one of the purified curare preparations to paralyze the respiratory muscles completely, after which he can be "breathed" with a mechanical respirator without difficulty. We have had no experience with this technic, but it seems logical and certainly deserves further study. Many years ago, in fact, in the first year a tank respirator was used, some success in this problem was achieved with the use of morphine in place of curare, although it was realized then that a "pure" curarelike action might be useful (Wilson, 1932)

Pulmonary Edema Pulmonary edema is a grave complication requiring emergency treatment. The mechanism of its production during acute poliomyelitis is still not clear. There is experimental evidence supporting the theory that involvement of certain portions of the midbrain may be primarily responsible This complication, when it arises, is always life-threatening, although it rarely lasts more than a few hours in those patients who survive Oxygen should be administered under pressure, either by face mask or tracheotomy tube attachment. If the patient is in a tank respirator the negative pressure should be increased to maximum. If signs of cardiac failure are present, standard treatment, including parenteral digitalization, is indicated If the patient has a tracheotomy, some physicians believe that a few drops of ethyl alcohol instilled directly into the trachea will decrease the foaminess of the edema fluid and allow better alveolar gas exchange

Hypertension. Mild hypertension fairly commonly accompanies poliomyelitis in the acute and early convalescent stages Its causes are still in dispute but are probably multiple Rarely, paroxysms of extremely severe hypertension are encountered These can be treated symptomatically with one of a number of autonomic blocking agents. However,

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acute phase of the disease and in early convalescence, but after weeks and months of this apprehensive, meticulous protection and isolation it is little wonder that some patients lose all incentive to live and families gradually drift away. Although convalescent polio patients may be severely handicapped physically and indeed some may be in a dangerous situation, they are not usually "sick" in the traditional sense of the word, and they need the stimulus of active and competitive living far more than the peace and the quiet of a standard hospital environment Where it is possible (see Respirator Centers) we believe that it is desirable to group as many as 10 or 20 patients in a single large room For the past several years we have mixed men, women and children in the same room without evidence of embarrassment and with clear evidence of the benefits gained by community living The patients are encouraged to wear slacks or shorts and sport shirts rather than the traditional hospital pajamas Standard beauty services should be available and used Visiting hours should be unlimited, except for short rest periods, in order to reestablish close family ties and to familiarize relatives with the details of necessary patient care Individual gains can be publicized, and the patient finds that, for the first time, he can compete on an equal basis with at least a few other people who have similar problems The patients also soon realize that they are not, and cannot be the center of attention They realize that there are other problems besides their own and that occasionally they must wait to get what they want It is also healthy, within limits, for a patient to learn to tolerate the activities of others even when these activities are annoying to him In other words, the willing acceptance of some irritating factors in his environment is essential for the mental attitude which enables a patient to strive aggressively to become again a useful citizen

Care of Patients with Maximum Paralysis in Respirator Centers. The long-time care of the severe paralytic patient is too complex a problem to be dealt with here in any detail, but a brief report of the development of the so-called Respirator Centers for the care of some of these patients is necessary

It became evident in the last 8 to 10 years that [illegible] the [illegible] with [illegible] lytic disease, had left in its wake a large number of terribly handicapped patients, living a life under practically custodial care in many hospitals, dependent upon this apparatus for their survival Accordingly, with aggressive action and money of the National Foundation for Infantile Paralysis, some 15 Respirator Centers for the care of these most terribly crippled patients were developed in the country Most of these patients not only had extensive paralysis of their respiratory muscles but also of their arms and legs It was evident that the mechanical respirator saves lives indeed, but often a life not worth saving unless some degree of self-sufficiency and independence can be developed It soon became apparent that much could be done even for the most tragically crippled patient The result of the work and the actions in these centers has been that a great many patients have been freed from immobilizing "respirator" care and have been returned to a life in their homes and often to useful occupation As an example of the last success, one center can report that of some 60 patients who had previously earned a living, 41 were returned to honest income-producing work even though some of them still required respiratory assistance The purpose of the centers, therefore, was (1) to free patients from respiratory assistance, particularly the immobilization of the tank respirator, (2) to return them to home life with continued medical and technical supervision when necessary, and (3) to give them assistance so that they could become useful or income-producing and, at the very least, care for themselves

There follow brief comments outlining the procedures for freeing a patient from immobilizing dependence on mechanical equipment

The respirator patient faces 3 handicaps

1 Most easily understood is the actual reduction of respiratory muscle capacity with resu[illegible]

2 [illegible] tion

reduction in elasticity of the thorax and the lungs and thus an increased resistance to expansion

preceded by a short period (from 30 minutes to 6 hours) of hypertension, and specific treatment of the hypertension with hypotensive drugs would be strongly contraindicated in these patients However, recurrent paroxysms of severe hypertension may in themselves be life-threatening and should be treated

Shock. The exact physiologic definition of shock is not agreed upon, but arterial hypotension seems to be a crucial factor. We know of no very effective method of treating "shock" occurring in acute poliomyelitis The majority of patients who develop cardiovascular collapse will die in spite of attempts at therapy However, some do survive, and the following measures are traditional and may be lifesaving. If the patient is in a tank respirator, the pressure setting should be altered so that approximately equal positive and negative pressures are used, thus helping to restore the normal filling pressure gradient in the right atrium. A few workers report favorable results from the use of norepinephrine by continuous intravenous drip Initial administration should be accompanied by almost continuous blood pressure measurements and the rate of administration adjusted to produce the desired blood pressure

Emotional Disturbance. The subject of treatment cannot be ended without some discussion of the care of the emotional disturbances which have been mentioned in general above The fact that severe emotional disturbances occur is very clear and does not seem difficult to understand. The physician must be aware that in part the widespread understanding and knowledge of the disease poliomyelitis and its well-advertised risks is going to cause a far greater fear of this disease than any other, except perhaps cancer. Immediately the diagnosis is entertained or made, some explanation must be given to any person of reasonable maturity that the degree of paralysis is by no means always great and that even when paralysis occurs, there may be a great deal of spontaneous recovery. The experience in handling badly paralyzed patients, particularly in our respirator centers, is great but does not lead to any possibility of a simple handling of the subject. Contacts of one patient with another equally seriously handicapped who has made considerable recovery and is accomplishing something with the aid of rehabilitation technics works wonders It was learned early in the respiratory centers that if the patients were together in one open ward the new victims were greatly encouraged by the progress that they could see made in their neighbors who had suffered the condition longer. Real hope given to a patient confined in a tank respirator that someday he can become independent even though handicapped, that he probably can be useful and even earn his own living, is the best form of therapy. The physician can help matters at the very beginning of the illness, particularly when it is apparent that the paralysis is going to be severe, by making the family also realize that they must keep a place for the patient in the home Often, when elaborate attempts at rehabilitation have been more or less successful, one finds the tragic situation that the home in the meanwhile has become closed. During the long periods of hospitalization, when the family did not really expect the patient to return, his place and space in the family life became occupied by another. This usually can be prevented Although suffering cannot be completely prevented by any means, a bad situation can be prevented from becoming worse if the physician is alert to the emotional consequences of the disease on the patient and his family at the onset of the disease.

Treatment of Emotional Problems Much can be done to foster good emotional hygiene in a convalescent poliomyelitis patient. Too often severely involved polio patients have been kept for months in quiet, private rooms, completely cut off from activities not essential for their immediate medical care Frequently, the patient's husband or wife may be able to visit for only an hour a day or perhaps only once or twice a week Often he is not allowed to see his children for months on end. Relatives are afraid to touch him or get near him for fear that something will happen and he will die The major emphasis is on immediate medical treatment, with any discussion of rehabilitation limited to general terms and projected to the future "when he gets better." Polio patients need the peace, the quiet, the sympathy and the protection from numerous visitors during the

and mice were susceptible to infection by poliomyelitis virus This discovery furnished far less expensive animals to work with, and quantitative studies of virus distribution and of antibodies could be undertaken Another important advance occurred when the chimpanzee was recognized as an animal which reacted most nearly like the human being and could be infected by oral administration of the virus.

Finally came what seems to be the greatest forward step of all, when the tissue culture technic for the study of poliomyelitis virus was made available by the studies of Enders and his associates (1949), so that really quantitative work could be carried out a hundred times more easily than in the early studies.

In the countries with the poorest sanitation, perhaps most of the inhabitants are infected very early. However, it is important to understand that the disease as we recognize it with its paralytic consequences occurs only rarely in these infections. To repeat, the paralytic disease is a rare complication of poliomyelitis virus infection

The natural spread of the virus in countries with poor hygiene is probably through the stools Most infants are infected in the early months of their lives when antibodies, transmitted from their mothers, still protect them so that they acquire active and permanent immunity during this safe period of passive immunity The disease is thus rare and is true "infantile paralysis" in primitive countries Only in more civilized countries in the last 50 years has the disease become epidemic, and during this period the total incidence of the disease has greatly increased During the same time the age incidence has shifted so that it is no longer truly "infantile" paralysis but a disease of children and young adults Table 14 indicates the reported incidence of the disease, but it must be remembered that such reports may be inaccurate (1) that mild cases are frequently missed, and (2) that nonparalytic poliomyelitis which swells the total reported incidence may not have been poliomyelitis at all but infection with some other virus (see Diagnosis).

In countries where epidemics occur, the virus is found to be widespread during these times, but it is not indiscriminately spread and is to be found more readily in people in close contact with patients The virus is excreted by infected individuals in the stools for various periods up to months, but also from the upper respiratory tract for short periods during the early acute febrile days of the disease Discussion as to which is the most important route for the spread of the virus in epidemic periods will be found in other sections of this chapter.

The epidemics occur in the late summer and early fall. No adequate explanation of this seasonal incidence has yet been accepted In the northern part of the United States the peak of the epidemic is in September,

in the United States, Sweden and Australia, though smaller epidemics are now being reported from nearly every country, those in the tropical world being less definite and severe In spite of the relative rarity of the paralytic disease, we must consider that the virus is highly communicable, being spread from one human being to another in a fairly direct fashion It is clear that man is the only host in which a sufficient number of individuals naturally support enough virus propagation to maintain the agent

History of Epidemiology

Early accounts of paralytic poliomyelitis describe it as a disease of young children, as the name "infantile paralysis" so clearly suggests, and sometimes it was also spoken of as "teething paralysis" Only 25 years ago it was stated by a few dogmatic clinicians that the safest place to be in a polio epidemic was in bed with another patient The belief that the disease was not contagious resulted from superficial observations at a time when the disease was not recognized except with

indicated the opposite and he himself suspected what we now know as the truth, that many people get infected without evident illness This concept had been clearly stated by Wickman and was frequently emphasized by the leading epidemiologists of the present generation: Frost, Aycock and Maxcy. But it was difficult for most people to grasp the idea that poliomyelitic infection

3. An emotional dependence on the respiratory equipment which may be very great indeed.

Various devices for respiratory aid exist in addition to the tank respirator, though all are less efficient than the "iron lung." The chest respirator is a cuirass type of device that fits only over the front of the abdomen and the chest but is operated on the same physical principles as the tank respirator It is light weight, the arms and the legs are free, and it can be used in a wheel chair with a battery-powered motor. The Rocking Bed makes use of the principle that with rhythmical rocking of a recumbent patient, the abdominal contents push first the diaphragm up in head-down position and then down in head-up position and produces a tidal exchange of air which, although not adequate for complete respiratory exchange, supplements the patient's own efforts

Another nonmechanical device was discovered almost by accident Certain patients learned "glossopharyngeal" respiration to carry out what has been called "frog breathing." This is a procedure in which by rhythmic swallowing motions the patient is able, with his pharyngeal muscles, to push air down into his lungs and inflate them to a marked degree. This is the actual mechanism of breathing of frogs Recognition of this procedure in certain patients who had discovered it spontaneously was reported by Dail et al (1955) and Collier et al (1956) The procedure was analyzed, and then it was found possible to teach it to many other patients These patients can inflate their lungs to a very satisfactory degree and under great pressure, in fact, sometimes the pressure is so great that it is possible to inhibit the return of blood to the right side of the heart, and fainting can result The "rate" of such glossopharyngeal respiration is slow, and the resultant gas exchange is not adequate for acute or severe paralysis, and of course it cannot permit sleep. Nevertheless, this procedure is entirely practical and has enabled many people to go many hours without mechanical assistance and during that time to be freed for training and for the actual accomplishment of self-care and income-producing activity.

The various rehabilitation technics for assistive apparatus in the use of the arms and the legs is in a stage of very active progress in this country and already has produced some most satisfactory results. The activities of the respirator centers probably furnish one of the most dramatic examples of what can be done by imaginative and devoted work in total patient care for handicapped individuals. Although such care is very expensive indeed, its total cost may well be considered returned from the saving of money for the costly hospitalization of these patients who otherwise would live indefinitely in hospitals in tank respirators

EPIDEMIOLOGY

A review of the development of knowledge of the epidemiology of poliomyelitis makes a fascinating study and, if complete, would include most of the scientific observations made of this disease until recent years. With the first understanding of the disease only paralytic cases could be recognized, and thus studies of epidemiology were limited. By some, infantile paralysis was not considered a contagious disease, although shrewd suspicions of others many years ago suggested that the paralytic cases could not represent the whole story and that the disease was indeed contagious It is convenient to divide the history of the studies of this disease into different periods, depending upon availability of technics for study In the first period only the "shoe-leather" technic was available; no laboratory procedures existed, and observations were limited to studying epidemiology as it appeared, with apparent paralytic disease the only indication of the action of the infectious agent. With only careful observation of nature, valuable studies were carried out by many very effective workers One of the earliest and greatest of the epidemiologists working with these handicaps was Wickman, to whom we shall refer again.

The next step, a crucially important one, was the determination by Landsteiner and Popper that this disease was indeed due to a virus, and that the rhesus monkey was a laboratory animal which could be used to detect the virus. Many years passed with patient but plodding progress under the handicap of restriction to the rhesus monkey as the only experimental host.

Another great advance occurred when Armstrong (1939) demonstrated that cotton rats

not produce immunity against one another. Although the blood serum of convalescents was known on occasion to contain antibody capable of neutralizing virus, this was not invariably true, a circumstance which led to much confusion

It fell to Burnet and MacNamara (1931) to observe that there were 2 types of convalescent sera, each of which neutralized different samples (strains) of virus, but the full meaning of this escaped them. Paul and Trask (1933) had produced more than one paralytic attack in one monkey, as had Howe and Bodian (1941a, 1942), but they also were groping in the dark.

The answer to the riddle came almost accidentally from the practice of using the Lansing strain for both vaccination and subsequent intracerebral challenge It soon became apparent that animals vaccinated with the Lansing strain and able to resist several hundred thousand ID_{50} nevertheless easily succumbed to much smaller challenges of the strain called "Brunhilde" The animals also failed to resist a strain obtained in California from a fatal case named Leon Furthermore, Lansing did not protect against Leon, and so on around the circle (Bodian et al, 1949, Kessel and Pait, 1949) So by this route came recognition of the 3 virus immunogenic types; Brunhilde (I), Lansing (II) and Leon (III). Extensive investigation of more than 100 other virus strains failed to reveal one which did not fit into one of the above categories (The Committee on Typing of the National Foundation for Infantile Paralysis, 1951)

A great discovery was made in the early 1940's when money from the March of Dimes made monkeys more readily available Polio virus was isolated from individual stools and from urban sewage (Paul and Trask, 1941) It was also recovered from filth and occasional house flies trapped in areas of ready access to exposed human feces (Trask et al, 1943, Trask and Paul, 1943) The demonstration of virus in the sewage of Toronto, Canada, and Johannesburg, S. A, during months when there were no reports of paralytic poliomyelitis furnished inescapable evidence of an immense reservoir of subclinical infection (Mundel et al, 1946; Rhodes et al, 1950)

Another outstanding step in the study of poliomyelitis was the use of the chimpanzee as the laboratory primate closest to man This animal has a broad susceptibility to polio viruses and, most important, is highly susceptible to poliomyelitis infection by the route presumably normal in humans, that is, by oral feeding (Howe and Bodian, 1941b; 1944; Howe et al, 1950). It was possible in these animals to measure the rise of virus titer in the infected alimentary tract and the corresponding serum antibody response. It was found that serum antibody titers of 1 to 300 or greater prevented *alimentary* infection entirely in the chimpanzee (Howe, 1955, 1957), but that lower levels allowed it to take place, thus converting the animal into a convalescent, but probably permanently immune to paralysis (Bodian, 1953) Paralysis was inhibited at much lower antibody levels and was not observed in any animal exhibiting definitely demonstrable antibodies (Bodian, 1952).

Change in Age Incidence

For the last 30 years, exposure to poliomyelitis virus has clearly become more difficult in early infancy, resulting in a relative shift in the paralytic disease to a progressively older age group (Dauer, 1955).

The early relative immunity of adults was always subject to discussion. It was thought that perhaps adults were simply enjoying one of their traditional prerogatives, that by simple physical maturity they became more refractory to paralysis. One answer lay in the [illegible]

disease in question was poliomyelitis. Often it seemed more likely that vitamin deficiencies, or some other condition was involved Finally, Type I poliovirus was identified from several fatal cases in a midwinter outbreak of a paralytic disease in an isolated group of Arctic Eskimos (Peart, 1949) These unfortunate people suffered attack rates of 20 to 25 per cent at all ages up to 60 years, thus indicating that their previous freedom from paralysis, for adults as well as children, was lack of exposure to the virus and that maturity per se played no part

During World War II, increased exposure was reflected by a 10-fold increase of paralytic rates among American troops stationed in Southeast Asia or the Eastern Mediterranean theaters as compared with those associated with duty in this country or continental Europe (Paul, 1949)

The habit of the disease, as time went on, to attack an older age group and to be epi-

was an unrecognized episode of childhood, as frequent as the flamboyant nuisance of measles, and resulting in lifelong freedom from the threat of paralysis.

Wickman was entirely ignorant of the etiologic agent and possessed no method of study except the observation of the human and clearly apparent disease, but he recognized it to be an infection communicated from one person to another He apparently was fortunate in being able to observe the action of a virulent virus strain which produced a high percentage of paralytic cases in small Swedish villages Such a situation enabled him to analyze the movements of people with enough accuracy to rule out common media such as food, milk, or water and to trace numerous chains of contact through paralytic cases Nonetheless, he recognized that there must also be many asymptomatic infections, although he could not estimate their relative frequency

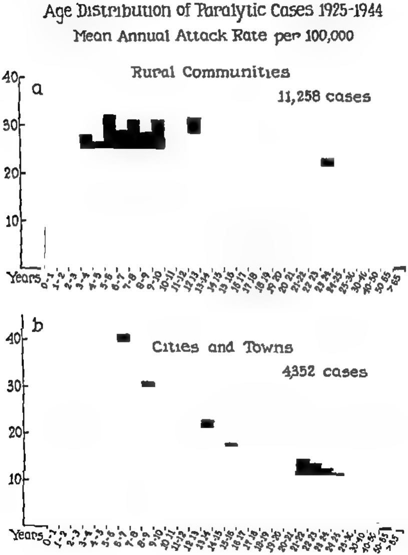
The first step that made it possible to use an animal host of the disease to study its nature began nearly 50 years ago with Landsteiner and Popper's success in causing a characteristic paralytic disease in the rhesus monkey At that time it was convincingly shown by the crude and expensive experiments made necessary by the limitation of the use of the rhesus monkey that neutralizing antibodies against the virus persisted for many years in convalescents from paralytic disease and were found also in those who had survived epidemics without suffering any apparent infection. Antibodies were believed to have intimate relationship to the lifelong immunity which seemed to follow paralysis, but cost prohibited the use of adequate numbers of animals for crucial studies to prove it Progress in knowledge was further handicapped by the fact that all strains of the virus were considered to be similar antigenically, and in fact only the Lansing strain (Type II) was in general experimental use in rhesus monkeys. With no knowledge that there were different antigenic types of polio virus, and since monkeys could sometimes be infected in whom antibodies had been demonstrated, it is not surprising that there was no agreement, even up until the beginning of the 1940's, as to the role of the circulating antibody in the prevention of paralysis Opinions ranged through every possible shade from no connection at all between antibodies and resistance to human disease to an all-important relationship.

The use of the rhesus monkey as an experimental animal was indeed a great advance However, the rhesus monkey did not provide a very good model for man, since this animal could be regularly infected with poliomyelitis only by direct contact between the virus and some exposed part of the nervous system. Nevertheless, much was learned Morgan and others (1947) found that if rhesus monkeys were vaccinated by large intramuscular injections, either with living or formalin-inactivated Lansing virus (Morgan, 1948), immunity to paralysis, from subsequent challenge, resulted with notable regularity. They were able to show that the serum antibody level rose in proportion to the amount of vaccine given and eventually attained titers of 1 to 1,000 or greater If the monkey possessed an antibody level of this magnitude at the time of intracerebral challenge, it always escaped paralysis, even though several hundred thousand doses of the same virus were injected directly into the brain (Morgan, 1949) Such a test was recognized to be far more drastic than any condition naturally occurring, but it clearly showed that the most susceptible tissue of the body could be protected from direct contact with virus if enough circulating antibodies were present.

For almost 30 years, in spite of devoted work, progress was greatly limited to the restrictions inherent in the fact that the rhesus monkey was the only experimental animal In 1939 Armstrong made another discovery of crucial importance He showed that he could cause paralytic disease in cotton rats and mice with injection of virus from monkey or man Again the handicap existed that the virus needed to be injected directly into the cerebrum. However, this success provided a new impetus to investigation by making available an inexpensive means for titrating antibodies and measuring virus with some accuracy This technic was used with profit in studying the propagation of the virus in these animals and the subsequent rise of antibodies The neutralization test carried out with the Lansing strain (Type II) against sera of unknown antiviral potency opened the way to an understanding of the minimal level of antibodies required to suppress virus growth by the combined use of the rhesus monkey and cotton rats and mice

It gradually became apparent that there were subgroups of poliomyelitis virus which were antigenically distinct and under artificial conditions in the laboratory, at least, did

Poliomyelitis is a disease which travels with the crowd, and frequently it has been shown that the acquisition of antibody or the incidence of paralytic disease is conditioned by the numbers and the propinquity of people living in a given area. The larger and more crowded the population, the younger the age at which either paralytic disease becomes manifest or antibody is acquired. Figure 58, taken from Olin (1952), contrasts the age incidence of carefully verified paralytic poliomyelitis cases in urban and rural areas of Sweden. This picture is repeated in any country where poliomyelitis is epidemic,



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demic has taken place in all the countries where poliomyelitis first ceased to be sporadic and demonstrates the consistence of the same initial forces which produced epidemics. It seems perhaps unfair at first to attribute this all to modern plumbing when nearly every phase of life is changed, but it was evident that the transfer of virus from pharynx to pharynx is less efficient in causing natural immunity than the ingestion of material contaminated by infected feces.

With better methods and less expensive ones being available for studying the distribution of virus and the presence of antibodies, epidemiologic studies of the disease became very significant when carried out elsewhere than in the modern countries where paralytic and epidemic poliomyelitis was common. One of the most revealing studies was that carried out in Cairo, Egypt, reported by Paul and his associates in 1952. They showed that most infants in Cairo, by the time they had reached the age of 2 years, had already acquired a significant level of antibodies to all 3 types, while in a city in a similar latitude and size in the United States only a very small percentage of children at this age had developed antibodies. It was noted that actual paralytic poliomyelitis probably occurred in Cairo much more often than had been reported or recognized, but it was evident that it was almost wholly "infantile" paralysis. The study was carried out with the use of mice for the detection and the measuring of virus and antibodies against the Lansing strain, Type II, but with the use of monkeys for the other types. The incidence of antibodies dropped off sharply in the early months of life but increased again sharply, so that roughly 80 per cent of the infants, in regard to some types, were immune or at least had antibodies at the age of 2 years.

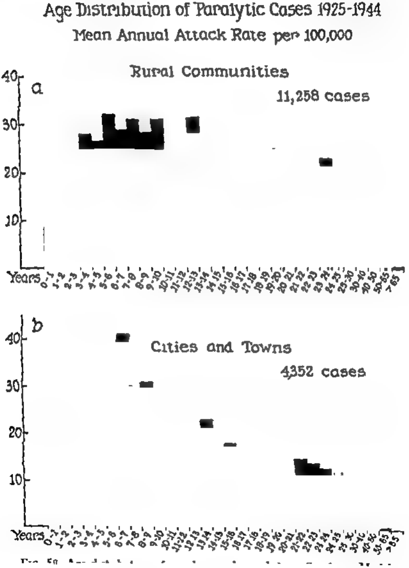
It became clear there that the virus was widespread in sewage and stools of many individuals not ill with the disease, and that natural infection in Egypt and other countries with similar poor sanitation, took place by the oral route without producing much disease, hardly any after infancy, but caused the development of permanent immunity in almost all young children.

The question arises as to why, if infection was widespread, the infants did not show a high incidence of paralytic disease in the process of developing immunity. Paralytic disease, true infantile paralysis, does occur in Egypt, but why is it not common? Data as to actual incidence are admittedly poor, but it seems certainly not great. There are two plausible explanations. Central nervous system disease from poliomyelitis seems to carry with it less risk of extensive paralysis in the young child than in the adult. This is evident in our own country where the severity of paralysis in adults who have central nervous system disease seems to be greater than that in children, if not infants. Another explanation seems to be highly probable. The natural immunity of the mother can be transferred to the infant through the placenta, as in the case of measles, and the newborn baby thus has passive antibodies which protect him for a number of months. It is probable that this passive immunity in the children of countries with poor sanitation protects the infant from paralytic disease while the infant is acquiring active immunity by the ingestion of potent viruses. It seems clear that in our country the price we pay for better sanitation, with its greatly reduced infant mortality from other conditions, is the failure of development of a natural immunity to poliomyelitis. Thus we have a logical explanation for the increased incidence of poliomyelitis in countries with better sanitation. This also explains the change in age incidence with the increasing involvement of older people that has been apparent in the last decade in this and European countries.

Spread of Virus. The presence of virus in the blood, the pharyngeal mucosa and its secretions, occasionally in saliva and the anterior part of the mouth, and lastly in the feces offers many possibilities for egress into the environment. It is now generally agreed that man is the only species in which the virus propagates; hence, he must be consid- [illegible] of virus

son-to-person transfer takes place without the intervention of any vector, even a blood-sucking arthropod. This latter possibility can be ruled out by a comparison of concurrent epidemics of western equine encephalomyelitis and poliomyelitis in Minnesota (Eklund, 1946). An epidemic of equine encephalomyelitis reached its peak in the month of July and then rapidly receded during August, which marks the beginning of colder weather in this state, and the disappearance of mosquitoes. At the same time an epidemic of poliomyelitis was just beginning and reached its maximum during the months of August and September but did not entirely subside until sometime in January.

Poliomyelitis is a disease which travels with the crowd, and frequently it has been shown that the acquisition of antibody or the incidence of paralytic disease is conditioned by the numbers and the propinquity of people living in a given area. The larger and more crowded the population, the younger the age at which either paralytic disease becomes manifest or antibody is acquired. Figure 58, taken from Olin (1952), contrasts the age incidence of carefully verified paralytic poliomyelitis cases in urban and rural areas of Sweden. This picture is repeated in any country where poliomyelitis is epidemic,



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although the differences between rural and urban areas grow less striking each year The presence of virus in the pharynx (Howe et al, 1945, Sabin, 1955) and the mouth, as well as in the feces (Trask et al, 1940), gives rise to a dilemma regarding the more important route by which it is transmitted from one person to another Every person infected with this virus for the first time, whether paralytic or asymptomatic, probably carries it in his throat for a period of 10 days or 2 weeks Some very young individuals also have virus in their mouths (Sabin, 1955, Howe, 1958), although the frequency and the duration of this occurrence is not yet determined Probably everyone experiencing a first infection sheds fecal virus for a week or more, but it persists in many during the 2nd to the 4th weeks (Horstmann et al, 1946, Schabel et al, 1950) and has been recorded as long as 84 to 123 days after the onset of infection (Goffe and Parfit, 1955, Lepine et al, 1939)

From an epidemiologic point of view there is reason to think that in the countries of the north and the south, so-called temperate zones, where poliomyelitis is epidemic, the fecal route may not be the all-important one, although it probably is in countries with more primitive types of sanitation

It is strikingly evident that the incidence of paralytic disease does not follow the pattern of enteric infections such as typhoid. The spread of the disease has seldom followed milk routes or water ways nor apparently often followed the "fingers, flies, feces" sequence of infection In the epidemic areas, evidence has been conspicuously lacking for dissemination of virus by any sort of common medium such as food or drink Water has never been incriminated (Maxcy, 1944), but a few milk-borne epidemics have been recorded These were traced to unpasteurized milk contaminated by an infected milker (Aycock, 1927, Dingman, 1916) Furthermore, secondary cases have not arisen from contact with known infected individuals during the long late stages of their disease, although many doubtless continue to eliminate fecal virus. On the contrary, there is striking agreement regarding the infectious period, as determined independently by 3 groups of [illegible] observing the incubation periods [illegible] case (Casey, [illegible] 1943; Silverthorne et al, 1949). The primary case appears to be most infectious from 7 to 10 days before and after the onset of symptoms. This corresponds very closely to the time during which virus may be demonstrated in the throat, although its duration is doubtless shorter in the mouth It was disappointing to learn that most family infections are nearly simultaneous rather than sequential with incubation periods of 7 to 14 days This was why gamma globulin given to contacts of the first patient in a family seldom has time to prevent other infections in that family.

One would naturally expect infection to reach its highest incidence in very young children, who are the most susceptible segment of the population. Extensive studies, now made possible in family groups by the use of tissue cultures, show beyond doubt that children under 5 years of age are probably the principal reservoirs of virus and the ones who introduce infection into the household. Anyone who has associated with children at this so-called "dirty age" can easily credit person-to-person transfer of virus from either end of the alimentary tract The study of Bodian and Paffenbarger (1954) also clearly indicates that a large proportion of so-called "index cases" in young adults probably were infected by their young children. On the contrary, Wehrle (1956) found no evidence of antibody rise in 50 hospital personnel (many without antibody of any type) during several weeks of exposure on acute poliomyelitis wards.

The Relationship of Artificial Immunization to the Spread of Virus

Discussion of the prevention of poliomyelitis by vaccine is given in Chapter 24 The influence of this mass immunization procedure on epidemiology is obviously of importance, and we must discuss briefly the relationship of artificial immunization to the spread of the virus.

The possibility that vaccination may reduce the duration of alimentary infection and limit its titer has important implications relative to the amount of virus in a community and to the passage of virus from one individual to another. If less virus were shed into the environment following vaccination it should be possible to immunize enough people to break the chain of transmission in certain areas where the virus of poliomyelitis would share the same fate as that of smallpox. However, it is very difficult to quantitate poliomyelitic infection without disease

in human beings, since many specimens are needed simply to measure its duration For

On the other hand, in chimpanzees it is possible to produce alimentary infection by the feeding of virus (Type I), after which one may obtain throat and mouth swabs, feces and blood daily for as long a period as desired Thus it is possible to observe the

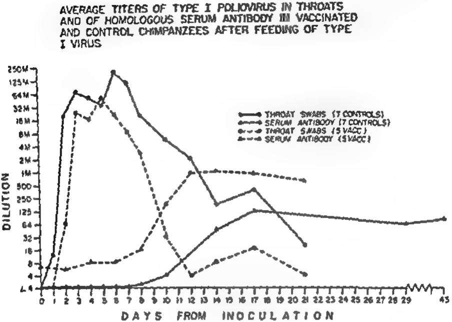

FIG 59A (Howe, H A, 1957, Day-by-day response of vaccinated chimpanzees to poliomyelitic infection, Am J Pub Health *47*, 871-875)

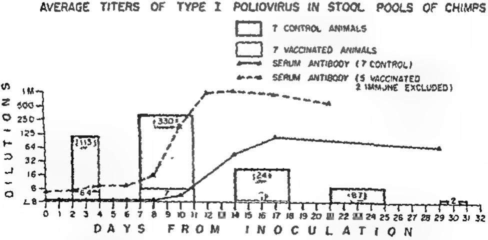

FIG 59B (Howe, H A, 1957, Day-by-day response of vaccinated chimpanzees to poliomyelitic infection, Am. J Pub Health *47*, 871-875)

duration and the amplitude of poliomyelitic infection at the above sites in relation to the rising titer of homologous serum neutralizing antibody (Howe, 1957). This is shown in Figure 59A for virus in the throat and in Figure 59B for virus in weekly stool pools. The titers represent an average of 5 vaccinated and 7 control (unvaccinated) animals. These Figures clearly show a consistent lowering of alimentary Type I titers, as well as a shortening of the carrier state in the vaccinated animals, as compared with the controls. Despite the intentionally low level of serum antibody in the vaccinated animals, they responded more rapidly and developed higher titers than the controls, but 2 vaccinated animals with high serum antibody titers at challenge are omitted, since they did not become infected. Virus titer declined as antibody rose with the 2 lines crossing at about the same point of equilibrium (1 to 64) in both groups of animals.

In a roughly comparable series of specimens that had been recently collected, on single occasions from paralytic patients and their family contacts, it is equally evident that virus titers in the throat, the mouth and even the stools bore an inverse relationship to the titers of homologous (Type I) serum antibody. However, stool virus was being excreted (at relatively low titer) in the presence of serum antibody levels above any observed to be compatible with the presence of virus in the throat or the mouth. While these observations are still fragmentary, they follow the same pattern as that observed in the chimpanzee except for the fact that in man the fecal virus titers are lower than the pharyngeal, just the reverse of the chimpanzee. For this reason it is doubtful whether the antibody levels achieved by vaccination would appreciably effect the elimination of fecal virus, a finding essentially in agreement with that of Fox (1957). Thus the epidemiologic pattern of poliomyelitis might show little change if the fecal route is of primary importance in virus dissemination. On the other hand, transmission of virus by the oropharyngeal route might be affected profoundly (Bell, 1948).

Actual observations of the effect of vaccination on the spread of virus are not yet clear. In the field study of the effect of vaccine, it was not apparent that nonparalytic

study. Moreover, it seems probable so far that the total reduction of poliomyelitis in 1956 and 1957 was greater than could be explained by the number of vaccinated individuals.

cial factor that makes the difference between infection and disease. What determines the "sorties" of the virus from its seat of innocent growth in the mucosa of the gastro-intestinal tract to the blood stream and the lymphatic channels, and, more specifically, what is the crucial factor that allows the virus to reach the central nervous system where its action causes the paralysis that makes the disease important?

We still are ignorant of one of the most striking characteristics of epidemics of poliomyelitis, that is, the seasonal incidence, which is in the summer and the early autumn in temperate zones. There has always been a suspicion that this could be related to an insect vector, but there is no support for this intriguing and at first thought logical hypothesis.

CONTROL

It seems clear that the virus of poliomyelitis is spread through the stools of infected individuals for considerable periods, and for short periods during the acute stages of the disease it is spread through the upper respiratory secretions. Attempts at quarantine have been undertaken for many years, and any outbreak of poliomyelitis presents a great problem to local health officers. One can emphatically challenge the success of any procedure used up to the present to control the spread of the disease. In the past, however, before knowledge of the spread of the virus was prevalent, isolation technics were more or less ignored in many places, though we had no evidence that the spread of disease was thereby greater. In large cities, patients with poliomyelitis are sent to isolation hospitals, but in many other areas they have been accepted for years in general hospitals without any specific or at least adequate isolation technics and with very little evidence of cross-infection. However, although there is little evidence of cross-infection of the disease within the hospital from patient to pa-

tient, there has been a considerable number of instances of transmission of the disease from patient to attendant, nurse or physician. From what has already been said about the virus in relationship to infection versus clinical disease, it becomes apparent why isolation technics might be ignored without evidence of any increased risk of the disease. The virus is widespread, therefore, isolation of a patient with clinical evidence of the disease succeeds at its best only in the prevention of the spread of a very tiny fraction of the virus excreted by the total number of infected individuals.

It seems certain that isolation technics have had little influence in controlling the spread of the disease, and that it is probably impractical ever to hope that the disease can be so controlled

The peak of incidence of the disease is in the late summer and fall There has been little evidence that closing swimming pools, delaying opening of schools, or any other public health procedures have actually influenced the incidence curve of the epidemic It is not apparent that a reduction in incidence in any epidemic occurring abruptly after some public health procedure has been instituted has actually occurred because of it. This, of course, cannot be used as an argument for abandonment of all attempts at isolation, and each community faced with an epidemic must of necessity institute what reasonable and practical steps are available

There is a great deal of question whether summer camps where poliomyelitis has occurred should be broken up and exposed children sent back to their homes where they may possibly further spread the virus However, pressure by parents usually demands this Justification for delaying the opening of a school is greatest where the starting of school would mean bringing back into a community a great many children who have been away all summer and thus scattered in various communities The closing of swimming pools is from one point of view a cheap procedure to carry out It causes only some disappointment, and it is difficult, in the face of an epidemic, for any public health organization to urge the continual use of public swimming pools where spread of enteric infection most logically could be aggravated

Many virus surveys show that the infection of one child in a family results in spread of the virus to practically all who are susceptible (Nolan et al, 1955; Wenner and Tanner, 1948, Gelfand et al, 1957; Howe, 1958). The evidence on this point is overwhelming, but the modest study by Goffe and Parfitt (1955) probably will deliver the *coup de grace* to the old notions of quarantine. In a small but modern English village (A) comprising 7,400 inhabitants, 2 cases of paralytic poliomyelitis occurred between January 8 and 13, 1954. There were also 3 cases among 96 persons residing in a nearby hamlet (B) which had no public water supply or sewage system The city fathers of "A" acted promptly, and on January 14 quarantined their cases Seven days later 8 out of 17 healthy household contacts were shown to have alimentary infections, and virus was found in sewage collected from a section of "A" quite remote from that of the "cases" Although the quarantine was still being maintained rigidly during the remainder of January, February and March, on March 2 a positive stool was found in 1 of 18 school children of "B"; and a collection of sewage from "A" on March 16 to 18 showed the presence of virus, even though there had been no recognized cases in this section at any time during the outbreak One stubborn contact continued to have positive stools for at least another month (beyond the 84th day)

Control by means of vaccines is discussed in detail in Chapter 24

REFERENCES

(For complete references, see also 2nd edition, Chapter 13)

Addair, J., and Snyder, L. H., 1942, Evidence for an autosomal recessive gene for susceptibility to

Anderson, G. W., Anderson, G., Skaar, A., and Sandler, F., 1952, Poliomyelitis in pregnancy, Am J Hyg *55*, 127-139

Armstrong, C., 1939, The experimental transmission of poliomyelitis to the eastern cotton-rat, Pub Health Rep *54*, 1719-1721

Aycock, W L, 1927, A milk-borne epidemic of poliomyelitis, Am J Hyg *7*, 791-803

Aycock, W. L., 1937, Nature of antarceologic susceptibility to poliomyelitis, Am J Pub Health *27*, 575-582

———, 1942, Tonsillectomy and poliomyelitis I Epidemiologic consideration Medicine *21*, 65-94

Aycock, W L., and Ingalls, T H, 1946, Abortion and polio, Am J M Sc *212*, 366-370

Aycock, W L., and Kessel, J F, 1943, The infectious period of poliomyelitis and virus detection, Am J M Sc *205*, 454-465

Aycock, W. L., et al, 1929, Preparalytic poliomyelitis, further observations on treatment with convalescent serum, J Infect Dis *45*, 175

Bahlke, A M, and Perkins, J E., 1945, Treatment of pre-paralytic poliomyelitis with gamma globulin, J A M A *129*, 1146 1150

Bell, E J, 1948, The relationship between the anti-poliomyelitic properties of human nasopharyngeal secretions and blood serums, Am J Hyg *47*, 351

Bhatt, P N, Brooks, M, and Fox, J P 1955, Extent of infection with poliomyelitis virus in household associates of clinical cases as determined serologically and by virus isolation using tissue culture methods, Am J Hyg *61*, 287-301

Bodian, D, 1949, Neutralization of three immunological types of poliomyelitis virus by human gamma globulin, Proc Soc Exper Biol & Med *72*, 259-261

———, 1952 Experimental studies on passive immunization against poliomyelitis II The prophylactic effect of human gamma globulin on paralytic poliomyelitis in cynomolgus monkeys after virus feeding Am J Hyg *56*, 78-89

———, 1953, Experimental studies on passive immunization against poliomyelitis III Passive-active immunization and pathogenesis after virus feeding in chimpanzees Am J Hyg *58*, 81-100

———, 1955, Viremia, invasiveness, and the influence of injections, Ann New York Acad Sc *61*, 877-882

———, 1956, Poliovirus in chimpanzee tissues after virus feeding, Am J Hyg *64*, 181-197

Bodian, D, Morgan, I M and Howe, H A, 1949, Differentiation of types of poliomyelitis viruses III The grouping of fourteen strains into three basic immunological types Am J Hyg *49*, 234-245

Bodian, D, and Paffenbarger, R S, Jr, 1954, Poliomyelitis infection in households frequency of viremia and specific antibody response, Am J Hyg *60*, 83-98

Brown, G C, Rabson, A S, and Schieble, J H, 1954, The effect of gamma globulin on subclinical infection in familial associates of poliomyelitis cases I Quantitative estimation of fecal virus J. Immunol *73*, 54-61

Brown, J R, and Baker, A. B, 1949, Poliomyelitis I Bulbar poliomyelitis A neurophysiological interpretation of the clinicopathological findings J Nerv & Ment Dis *109*, 54

Burnet, F M, and MacNamara, J, 1931, Immuno- [illegible] strains of poliomyelitic [illegible]

C[illegible] in epi-

———, 1942b, Observations on an epidemic of poliomyelitis, Science *95*, 359-360

Casey, A E, Fishbein, W. I, Schabel, F. M, Jr, and Smith, H. T. 1950, Incidence of subclinical poliomyelitis in an urban area according to age groups, Am J Pub Health *40*, 1241-1250

Collier, C R, Dail, C W, and Affeldt, J E, 1956, Mechanics of glossopharyngeal breathing, J Appl Physiol *8*, 580-584

Colter, J S, Bird, H H, Moyer, A W, and Brown, R. A, 1957, Infectivity of ribonucleic acid isolated from virus-infected tissues, Virology *4*, 522-532

The Committee on Typing of the National Foundation for Infantile Paralysis, 1951, Immunologic classification of poliomyelitis viruses I A cooperative program for the typing of 100 strains (and 8 other papers on typing) to V Discussion of results of General Summary Am J. Hyg *54*, 191-274

Copellman, F S, 1944, Follow-up of 100 children with poliomyelitis, Family *25*, 289-297

Dail, C W, Affeldt, J E, and Collier, C. R, 1955, Clinical aspects of glossopharyngeal breathing, report of use by 100 postpoliomyelitic patients, J A M A *158*, 445-449

Dauer, C C, 1955, The changing age distribution of paralytic poliomyelitis the biology of poliomyelitis, Ann New York Acad. Sc *61*, 943-955

Dick, G W A, Dane, D S, Fisher, O D, Con- [illegible] 1957 Vaccination [illegible] rus vaccine Brit M J [illegible]

Dick, G W A, Schwerdt, C E, Huber, W, Sharpless, G. R, and Howe, H A, 1951, Immunization of cotton rats with inactivated Lansing poliomyelitis virus II Inactivation by physical methods Am J Hyg *53*, 131-138

Dickinson, D G, Wilson, J L, and Graham, B D, 1953, Studies in respiratory insufficiency I Carbon dioxide and oxygen studies in early respiratory paralysis in poliomyelitis A M A Am J Dis Child *86*, 265-272

Dingman, J C, 1916, Report of a possibly milk-borne epidemic of infantile paralysis, New York State J Med *16*, 389-390

Doll, R., and Hill, A B, 1957, Deaths from poliomyelitis among British doctors, Brit M J *1*, 372

Dulbecco, R, and Vogt, M, 1955, Biological properties of poliomyelitis viruses studied by the Plaque technique biology of poliomyelitis, Ann New York Acad Sc *61*, 790-800

Dunham, W B, and Ewing, F. M, 1957, Propagation of poliovirus in chick embryo cell cultures I Cultivation of 3 virus types Proc Soc Exper Biol & Med *95*, 637-639.

Eklund, C M, 1946, Human encephalitis of the Western equine type in Minnesota in 1941 clinical and epidemiological study of serologically positive cases, Am J. Hyg *43*, 171-193.

Elford, W J, Galloway, I A, and Perdrau, J R, 1935, The size of the virus of poliomyelitis as determined by ultrafiltration analysis, J Path & Bact *40*, 135-140

Enders, J F, Weller, T H, and Robbins, F C, 1949, Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues, Science *109*, 85-87

Engstrom, C G, 1954, Treatment of severe cases of respiratory paralysis by Engstrom universal respirator, Brit M J *2*, 666-669

Finn, S, Korns, R F, and Bahlke, A M, 1947, Exposed dental pulp as a portal of entry for the virus of poliomyelitis, Am J Hyg *46*, 177-184

Foster, C, Jones, J H, Henle, W, and Dorfman, F, 1943, Relation of food intake to response of mice inoculated with Lansing strain of Murine poliomyelitis virus, Science *97*, 207-208

Fox J P, 1958, Epidemiology of poliomyelitis in populations before and after vaccination *in* Poliomyelitis Papers and Discussions Presented at the Fourth International Polio Conference, Philadelphia, Lippincott

Francis, T, Jr et al, 1957, Evaluation of the 1954 field trial of poliomyelitis vaccine (final report) (cf Chap 24 for complete reference)

Gelfand, H M, LeBlanc, D R, Fox, J P, and Conwell, D P, 1957, Studies on the development of natural immunity to poliomyelitis in Louisiana II Description and analysis of episodes of infection observed in study group households Am J Hyg *65*, 367-385

Gershoff, S N, Rasmussen, A F, Jr, Elvehjem, C A, and Clark, P F, 1952 Effect of amino acid imbalance on course of Lansing Poliomyelitis in mice Proc Soc Exper Biol & Med *81*, 484-486

Glaser R F, 1945, Tracheotomy in bulbar poliomyelitis J Pediat 27, 360

Goffe A P, and Parfitt E M 1955, Quarantine measures in poliomyelitis, Lancet *1*, 1172 1175

Grossman H J, Gibbs, E L, and Spies, H W, Electroencephalographic studies on patients having poliomyelitis with no clinical evidence of encephalitic involvement Unpublished

Harmon, P H, 1934, Poliomyelitis, experimental and theoretical basis for serum therapy, review, Am J Dis Child *47*, 1179, Poliomyelitis, results of treatment in acute disease, analysis of reports on 4,400 patients treated with serum, observations of 2,660 untreated patients, Am J Dis Child *47*, 1216

Henjesh, M, Pollard, E C, Opton, E M, Black, F L Bellamy, D, and Melnick, J, 1958, Size and structure of ECHO, poliomyelitis, and measles viruses determined by ionizing radiation and ultra filtration, Virology In press

Herndon, C N, and Jennings, R G, 1951, A twin-family study of susceptibility to poliomyelitis, Am J Human Genet *3*, 17-46

Hill, A B, and Knowelden, J, 1950, Inoculation and poliomyelitis, a statistical investigation in England and Wales in 1949, Brit M J *2*, 1-6

Holmgren, B E, 1952, Electro-encephalography in poliomyelitis *in* Poliomyelitis Papers and Discussions Presented at Second International Poliomyelitis Conference, pp 448-452, Philadelphia, Lippincott

Horstmann, D M, 1949, Clinical aspects of acute poliomyelitis, Am J Med *6*, 592-605

———, 1950, Acute poliomyelitis relation of physical activity at the time of onset to the course of the disease, J A M A *142*, 236-241

Horstmann, D M, McCollum, R W, and Mascola, A D, 1954, Viremia in human poliomyelitis, J Exper Med *99*, 355-370

Horstmann, D M, Ward, R, and Melnick, J L, 1946, The isolation of poliomyelitis virus from human extra-neural sources III Persistence of virus in stools after acute infection J Clin Invest *25*, 275-277

Howe, H A, 1949, The epidemiology of poliomyelitis in the light of modern research, Am J Med *6*, 537-550

———, 1954, Studies of active immunogenesis in poliomyelitis I Persistence and recall by homotypic or heterotypic superinfection of neutralizing antibody originally induced in chimpanzees by vaccination or infection Am J Hyg *60*, 371-391

———, 1955, Poliomyelitis infection in immunized chimpanzees, Ann New York Acad Sc *61*, 1014-1022

———, 1957, Day-by-day response of vaccinated chimpanzees to poliomyelitic infection, Am J Pub Health *47*, 871-875

———, 1958, A relationship between serum antibody titer and the titer of virus in throat swabs, mouth swabs and stools of poliomyelitis patients and their family contacts In preparation

Howe, H A, and Bodian, D, 1941a, Second attacks of poliomyelitis an experimental study J Exper Med *74*, 145-166

——— 1941b, Poliomyelitis in the chimpanzee a clinical pathological study, Bull Johns Hopkins Hosp *69*, 149-181

———, 1942, Neural Mechanisms in Poliomyelitis, Chap 10, New York, Commonwealth Fund

——— 1944, Poliomyelitis by accidental contagion in the chimpanzee, J Exper Med *80*, 383-390

———, 1947, Isolation of poliomyelitis virus from the throats of symptomless children, Am J Hyg *45*, 219-222

Howe, H A, Bodian, D, and Morgan, I M, 1950, Subclinical poliomyelitis in the chimpanzee and its relation to alimentary reinfection, Am J Hyg *51*, 85-108

Howe, H A, Bodian, D, and Wenner, H A, 1945, Further observations on the presence of poliomyelitis virus in the human oropharynx, Bull Johns Hopkins Hosp *76*, 19-24

International Poliomyelitis Congress, 1949, Poliomyelitis Papers and Discussions Presented at the First International Poliomyelitis Conference, Philadelphia, Lippincott

International Poliomyelitis Congress, 1952, Poliomyelitis Papers and Discussions Presented at the Second International Poliomyelitis Conference, Philadelphia, Lippincott

International Poliomyelitis Congress, 1955, Poliomyelitis Papers and Discussions Presented at the Third International Poliomyelitis Conference, Philadelphia, Lippincott

Jungeblut, C W, 1937, Effect of ultraviolet irradiation on poliomyelitis virus in vitro, Proc Soc Exper Biol & Med *37*, 160-162

Aycock, W L, 1937, Nature of antarceologic susceptibility to poliomyelitis, Am J Pub Health 27, 575-582

———, 1942, Tonsillectomy and poliomyelitis I Epidemiologic consideration Medicine 21, 65-94

Aycock, W L, and Ingalls, T H, 1946, Abortion and polio, Am J M Sc 212, 366-379

Aycock, W L, and Kessel, J F, 1943, The infectious period of poliomyelitis and virus detection, Am J M Sc 205, 454-465

Aycock, W L, et al, 1929, Preparalytic poliomyelitis, further observations on treatment with convalescent serum, J Infect Dis 45, 175

Bahlke, A M, and Perkins, J E, 1945, Treatment of pre-paralytic poliomyelitis with gamma globulin, J A M A 129, 1146-1150

Bell, E J, 1948, The relationship between the anti-poliomyelitic properties of human nasopharyngeal secretions and blood serums, Am J Hyg 47, 351

Bhatt, P N, Brooks, M, and Fox, J P, 1955, Extent of infection with poliomyelitis virus in household associates of clinical cases as determined serologically and by virus isolation using tissue culture methods, Am J Hyg 61, 287-301

Bodian, D, 1949, Neutralization of three immunological types of poliomyelitis virus by human gamma globulin, Proc Soc Exper Biol & Med 72, 259-261

———, 1952, Experimental studies on passive immunization against poliomyelitis II The prophylactic effect of human gamma globulin on paralytic poliomyelitis in cynomolgus monkeys after virus feeding Am J Hyg 56, 78 89

———, 1953, Experimental studies on passive immunization against poliomyelitis III Passive-active immunization and pathogenesis after virus feeding in chimpanzees Am J Hyg 58, 81-100

———, 1955, Viremia, invasiveness, and the influence of injections, Ann New York Acad Sc 61, 877-882

———, 1956, Poliovirus in chimpanzee tissues after virus feeding, Am J Hyg 64, 181-197

Bodian, D, Morgan, I M and Howe, H A, 1949, Differentiation of types of poliomyelitis viruses III The grouping of fourteen strains into three basic immunological types Am J Hyg 49, 234-245

Bodian, D, and Paffenbarger, R S, Jr, 1954, Poliomyelitis infection in households frequency of viremia and specific antibody response, Am J Hyg 60, 83-98

Brown, G C, Rabson, A S, and Schieble, J H, 1954, The effect of gamma globulin on subclinical infection in familial associates of poliomyelitis cases I Quantitative estimation of fecal virus J. Immunol 73, 54-61

Brown, J R., and Baker, A B, 1949, Poliomyelitis I Bulbar poliomyelitis A neurophysiological interpretation of the clinicopathological findings J Nerv & Ment Dis 109, 54

Burnet, F M, and MacNamara, J, 1931, Immuno-... differences between strains of poliomyelitic

C... in epi-

———, 1942b, Observations on an epidemic of poliomyelitis, Science 95, 359-360

Casey, A E, Fishbein, W I, Schabel, F. M., Jr., and Smith, H T, 1950, Incidence of subclinical poliomyelitis in an urban area according to age groups, Am J. Pub Health 40, 1241-1250

Collier, C. R, Dail, C. W, and Affeldt, J. E, 1956, Mechanics of glossopharyngeal breathing, J Appl Physiol 8, 580-584

Colter, J S, Bird, H H, Moyer, A W., and Brown, R A, 1957, Infectivity of ribonucleic acid isolated from virus-infected tissues, Virology 4, 522-532

The Committee on Typing of the National Foundation for Infantile Paralysis, 1951, Immunologic classification of poliomyelitis viruses I A cooperative program for the typing of 100 strains (and 8 other papers on typing) to V. Discussion of results of General Summary Am J Hyg 54, 191-274

Copellman, F S, 1944, Follow-up of 100 children with poliomyelitis, Family 25, 289-297

Dail, C W, Affeldt, J E, and Collier, C R., 1955, Clinical aspects of glossopharyngeal breathing, report of use by 100 postpoliomyelitic patients J A M A 158, 445-449.

Dauer, C C., 1955, The changing age distribution of paralytic poliomyelitis the biology of poliomyelitis, Ann New York Acad Sc 61, 943-955

Dick, G W A, Dane, D S, Fisher, O D., Connolly, J H, and McKeown, F., 1957, Vaccination against poliomyelitis with live virus vaccines 2 A trial of SM Type I attenuated poliomyelitis virus vaccine Brit M J, 1, 65-70

Dick, G W A, Schwerdt, C E, Huber, W, Sharpless, G R, and Howe, H A, 1951, Immunization of cotton rats with inactivated Lansing poliomyelitis virus II Inactivation by physical methods Am J Hyg 53, 131-135

Dickinson, D G., Wilson, J L, and Graham, B D., 1953, Studies in respiratory insufficiency I Carbon dioxide and oxygen studies in early respiratory paralysis in poliomyelitis A M A Am J Dis Child 86, 265-272

Dingman, J C, 1916, Report of a possibly milk borne epidemic of infantile paralysis, New York State J Med. 16, 589-590.

Doll, R, and Hill, A B, 1957, Deaths from poliomyelitis among British doctors, Brit M J 1, 372

Dulbecco, R, and Vogt, M, 1955, Biological properties of poliomyelitis viruses studied by the Plaque technique biology of poliomyelitis, Ann New York Acad Sc 61, 790-800

Dunham, W B, and Ewing, F M, 1957, Propagation of poliovirus in chick embryo cell cultures I Cultivation of 3 virus types Proc Soc Exper Biol & Med 95, 637-639.

Eklund, C M, 1946, Human encephalitis of the Western equine type in Minnesota in 1941 clinical and epidemiological study of serologically positive cases, Am J. Hyg 43, 171-193

Elford, W J, Galloway, I A, and Perdrau, J R., 1935, The size of the virus of poliomyelitis as determined by ultrafiltration analysis, J Path & Bact 40, 135-140

in Canadian Eskimos in wintertime, Canad. J Pub Health *40*, 405-417

Plum, F., and Dunning, M F., 1956, Techniques for minimizing trauma to the tracheo bronchial tree following tracheotomy, New England J Med *254*, 193

Pohl, J F., 1945, The Kenny Concept and treatment of infantile paralysis Report of five year study of cases treated and supervised by Miss Elizabeth Kenny in America Lancet *65*, 265

Pohl, J F., and Kenny, E., 1943, The Kenny Concept of Infantile Paralysis and its Treatment, St. Paul, Minn, Bruce Pub Co.

Pollard, M., and Connolly, J, 1949, pH stability of MEF poliomyelitis Texas Rep Biol & Med *7*, 92-95

Rhodes, A J., Clark, E M., Knowles, D S., Shimada, F., Goodfellow, A M., Ritchie R C., and Donohue, W L., 1950, Poliomyelitis virus in urban sewage an examination for its presence over a period of twelve months, Canad J Pub Health *41*, 248-254

Rhodes, A J., Clark, E M., Knowles, D S., Shimada, F T, Ritchie, R C., Donohue, W L., Armstrong, M P., Wilson, F H., McLean, W J., and Silverthorne, N., 1950, Studies on poliomyelitis in Ontario III Further observations on the association of Coxsackie and poliomyelitis viruses Canad. J Pub Health *41*, 183-188

Roca-Garcia, M, and Jervis, G A., 1955 Experimentally produced poliomyelitis in chick embryo, Ann New York Acad. Sc *61*, 911-923

Rosen, L., and Thooris, G., 1953, Poliomyelitis in French Oceania. Epidemiologic observations on an outbreak with notes on the incidence of paralysis following intramuscular injections Am. J Hyg *57*, 237-252

Russell, W R, 1947, Poliomyelitis, pre-paralytic stage and effect of physical activity on severity of paralysis, Brit M J *2*, 1023

——, 1949, Paralytic poliomyelitis. The early symptoms and the effect of physical activity on the course of the disease Brit M J *1*, 465

——, 1952, Poliomyelitis, Edinburgh M. J *59*, 530

Sabin, A B., 1950, Relative resistance of newborn mice to poliomyelitis virus, Proc Soc Exper Biol & Med *73*, 394-399

——, 1955, Quantitation of virus in stools, throats, mouth and nose of poliomyelitis patients in relation to serum antibody titers Personal communication

——, 1956, Pathogenesis of poliomyelitis, Science *123*, 1151-1157

——, 1957, Properties and behavior of orally administered attenuated poliovirus vaccine, J.A.M.A *164*, 1216 1223

Sabin, A B., Hennessen, W A., and Warren, J., 1954, Ultrafiltration and electron microscopy of three types of poliomyelitis virus propagated in tissue culture, Soc Exper Biol. & Med *85*, 359-363.

Sabin, A B, and Ward, R., 1941, The natural history of human poliomyelitis. I Distribution of virus in nervous and non nervous tissues J Exper Med. *73*, 771-793.

Salk, J E., Youngner, J. S., and Ward, E. N., 1954, Use of color change of phenol red as the indicator in titrating poliomyelitis virus or its antibody in a tissue-culture system, Am J Hyg *60*, 214-230

Sarnoff, S J., Hardenbergh, E., Whittenberger, J L., 1948, Electrophrenic respiration, Am J Physiol. *155*, 1-9

Sarnoff, S J., Maloney, J. V., Jr., Sarnoff, L. C., Ferris, B G., Jr., and Whittenberger, J. L., 1950, Electrophrenic respiration in acute bulbar poliomyelitis. Its use in management of respiratory irregularities. J.A.M.A *143*, 1383-1390

Schabel, F. M., Jr., Smith, H T., Fishbein, W. I., and Casey, A. E., 1950, Stool virus recovery in subclinical poliomyelitis during incubation, febrile, and convalescent periods, J Infect Dis. *85*, 214-218

Scherer, W F., 1955, Comparative susceptibility of cells of the same type to infection by poliomyelitis virus biol of poliomyelitis, Ann New York Acad Sc *61*, 806-821

Schwerdt, C. E., and Fogh, J., 1957, The ratio of physical particles per infectious unit observed for poliomyelitis viruses, Virology *4*, 41-52

Schwerdt, C. E., and Schaffer, F L., 1955, Some physical and chemical properties of purified poliomyelitis virus preparations biol of poliomyelitis, Ann New York Acad. Sc. *61*, 740-750

Sheffield, F W., and Churcher, G M., 1957, The serial propagation of poliomyelitis viruses in cells derived from rabbit embryo kidney, Brit J Exper Path *38*, 155

Silverthorne, N., Armstrong, M P., Wilson, F H., Donohue, W L., Goodfellow, A M., Roy, T. E., McLelland, L., Clark, E M., and Rhodes, A J., 1949, Studies on poliomyelitis in Ontario I Observations on the apparent infectiousness of the acute case Canad. M A J *61*, 241-250

Sprunt, K., Mountain, I. M., Redman, W M., and Alexander, H. E, 1955, Production of poliomyelitis virus with combined antigenic characteristics of type I and type II Virology *1*, 236-249

Top, F H, 1952, Occurrence of poliomyelitis in relation to tonsillectomies at various intervals, J.A.M.A *150*, 534-538

Trask, J D., and Paul, J R., 1943, The detection of poliomyelitis virus in flies collected during epidemics of poliomyelitis. II Clinical circumstances under which flies were collected J Exper Med *77*, 545-556

Trask, J D, Paul J R., and Melnick, J L., 1943, The detection of poliomyelitis virus in flies collected during epidemics of poliomyelitis. I. Methods, results, and types of flies involved J Exper Med *77*, 531 544

Trask, J. D., Paul, J R., Vignec, A J., 1940 I Poliomyelitic virus in human stools, J Exper Med *71*, 751-763.

Webster, L T., 1937, Inheritance of resistance of

Kaplan, A S, and Melnick, J. L, 1952, Effect of milk and cream on the thermal inactivation of human poliomyelitis virus, Am J Pub Health *42*, 525-534

Kessel, J F, and Pait, C F, 1949, Differentiation of three groups of poliomyelitis virus, Proc Soc Exper Biol & Med *70*, 315-316

Koprowski, H, Norton, T W, and McDermott, W., 1947, Isolation of poliomyelitis virus from human serum by direct inoculation into a laboratory mouse, Pub Health Rep *62*, 1467-1498

Koprowski, H, Norton, T W, Jervis, G A, Nelson, T L, Chadwick, D L, Nelsen, D J, and Meyer, K F, 1956, Clinical investigations on attenuated strains of poliomyelitis virus Use as a method of immunization of children with living virus J A M A *160*, 954-966

Korns, [illegible] F, Albrecht, R M, and Locke, F B, 1952, The association of parenteral injections with poliomyelitis, Am J Pub Health *42*, 153-169

Kramer, [illegible] D, Aycock, W L, Solomon, C I, and *Thenebe, C L, 1932, Convalescent serum therapy* in preparalytic poliomyelitis, New England J Med *206*, 432

Krech, U, 1954a, Intracerebral adaptation to mice of the Leon strain of type 3 poliomyelitis virus, Proc Soc Exper Biol & Med *85*, 344-346

———, 1954b, Susceptibility of mice to infection with Mahoney strain of type I poliomyelitis virus, Proc Soc Exper Biol & Med *86*, 192-194

Landry, J, 1859, Note sur la paralysie ascendante aigue, Gaz hebd de méd *6*, 472-474 (July 9), 486 488 (August 5) (Acute ascending paralysis)

Lensen, S G, Rhian, M, Stebbins, M R, Backus, [illegible] C, and Peterson, C E, 1949, Inactivation of partially purified poliomyelitis virus in water by chlorination, experiments with natural waters, Am J Pub Health *39*, 1120-1128 Bibliog of Inf Paral, 714, 1789 1949

Lepine, P, Sedallian, P, and Sautter, V, 1939, Sur la presence du virus poliomyelitique dans les matieres fecales et sa longue duree d'elimination chez un porteur sain, Bull Acad Med Paris. *122*, 141-149

Levinson, S O, Milzer A, and Lewin, P, 1945, Effect of fatigue, chilling and mechanical trauma on resistance to experimental poliomyelitis, Am J Hyg *42*, 204

Li, C P, and Habel, K, 1951, Adaptation of Leon strain of poliomyelitis to mice, Proc Soc Exper Biol & Med *78*, 233-238

Li, C P, and Schaeffer, M, 1953, *Adaptation of* type I poliomyelitis virus to mice, Proc Soc Exper Biol & Med *82*, 477-481

McCloskey, B P, 1950, The relation of prophylactic inoculations to the onset of poliomyelitis, Lancet *1*, 659-663

Maxcy, K F, 1944, A review of epidemiology of acute anterior poliomyelitis with reference to the mode of transmission, Lancet *64*, 216-223

Melnick, J. L., and Horstmann, D M, 1947, Active immunity to poliomyelitis in chimpanzees following subclinical infection, J Exper Med. *85*, 287-303

Melnick, J L., Kaplan, A S, Zabin, E, Contreras, G, and Ledinko, N W., 1951, An epidemic of paralytic poliomyelitis characterized by dual infections with poliomyelitis and Coxsackie viruses, J Exper. Med *94*, 471-492

Melnick, J. L, and Ledinko, N., 1952, Vaccination as a provoking factor in poliomyelitis an experimental approach, J Infect Dis *90*, 279-282

Morch, E T., Saxton, G A., and Gish, G., 1956, Artificial respiration via the uncuffed tracheostomy tube, J.A.M.A *160*, 864

Morgan, I M., 1948, Immunization of monkeys with formalin-inactivated poliomyelitis viruses, Am J Hyg *48*, 394-406

———, 1949, Level of serum antibody associated with intracerebral immunity in monkeys vaccinated with Lansing poliomyelitis virus, J Immunol *62*, 301-310

Morgan, I M, Howe, H A, and Bodian, D, 1947, *The role of antibody in experimental poliomyelitis* II Production of intracerebral immunity in monkeys by vaccination Am J Hyg *45*, 379-389

Mundel, B., Gear, J H S, and Wilson, D, 1946, Studies in poliomyelitis Distribution of virus of poliomyelitis in sewage purification works in Johannesburg South African M J *20*, 336-338

Nolan, J P, Wilmer, B H, and Melnick, J L., 1955, Poliomyelitis *Its highly invasive nature and narrow stream of infection in a community of high socio-economic level*, New England J Med *253*, 945-954

Olin, G, 1952, The epidemiologic pattern of poliomyelitis in Sweden from 1905 to 1950 *in* Poliomyelitis Papers and Discussions Presented at the Second International Poliomyelitis Conference, pp 367-375, Philadelphia, Lippincott

Paffenbarger, R S, Jr, and Wilson, V. O, 1955, Previous tonsillectomy and current pregnancy as they affect risk of poliomyelitis attack, Ann New York Acad Sc *61*, 856-868

Paffenbarger, R S, Jr, Wilson, V O, Bodian, D, and Watt, J, 1954, The spread of poliomyelitis an analysis of contact during epidemic periods, Am J Hyg *60*, 63-82

Park, W H, 1932, Therapeutic use of antipoliomyelitis serum in preparalytic cases of poliomyelitis J.A M.A *99*, 1050

Paul, J R, 1949, Poliomyelitis attack rates in American troops, 1940-1948, Am J Hyg *50*, 57-62

Paul, J R, Melnick, J L, Barnett, V. H, and Goldblum, N, 1952, Survey of neutralizing antibodies to poliomyelitis virus in Cairo, Egypt. Am J Hyg *55*, 402.

Paul, J R, and Trask, J D, 1933, A comparative study of recently isolated human strains and a passage strain of poliomyelitis, J Exper Med *58*, 513-530

Paul, J R, and Trask, J. D., 1941, The virus of *poliomyelitis in stools and in sewage*, J.A M.A *116*, 493, 497

Peart, A F. W, 1949, An outbreak of poliomyelitis

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23

Poliomyelitis: Pathogenesis and Histopathology

INTRODUCTION

The nature of a viral infection is determined by both stable and variable properties of the viral and host cell reacting system. In very few viral infections are the host variables so numerous and so complex as in poliomyelitis. This disease is an occasional neuropathologic resultant of a sequence of relatively silent physiologic events of infection which occur in most members of the human species at least once in their lifetimes. Understanding of the silent as well as the overt events of infection has increased greatly in recent years, although several basic problems are still unresolved. Nevertheless, present knowledge of the pathogenesis of this disease makes possible considerable insight into the factors which determine the outcome of infection, as well as those which are the basis of diagnosis, treatment, or prevention. The intimate details of the interplay of these factors can be approached best after consideration of the sequence of events in natural infections.

PORTAL OF ENTRY AND EXIT OF VIRUS

Epidemiologic evidence has led to the inescapable conclusion that the virus of poliomyelitis is spread directly or indirectly from person to person. No intermediate host is known. The crucial role of a human reservoir of infection is supported by the finding of virus in oropharyngeal secretions and in feces well before onset of symptoms or of other signs of infection. Although the precise details of the manner of spread of infection from person to person are not known, it is now generally believed that entry of virus is by way of the upper alimentary tract, and exit by way of the upper and the lower alimentary portals. The previously held belief that virus enters by the nasal route has been abandoned.

PRIMARY VIRAL IMPLANTATION AND PRIMARY SITES OF VIRAL MULTIPLICATION

The early stages of infection in poliomyelitis were largely unknown or misunderstood before experimental work with freshly isolated strains began on an experimental model, the chimpanzee, which is the anthropoid ape most closely related phylogenetically to the human species. The responses of the chimpanzee after virus feeding are indistinguishable from those of human beings with respect to observable events of infection (Bodian, 1952, 1955), and recently the similarity of the infection has been confirmed by studies of the course of infection in human beings

mice to enteric bacterial and neurotropic virus infections, J Exper Med 65, 261-286

Wehrle, P F, 1956, The risk of poliomyelitis infection among exposed hospital personnel, Pediatrics 17, 237-246

Weinstein, L, 1957, Influence of age and sex on susceptibility and clinical manifestations in poliomyelitis, New England J Med 257, 47-52

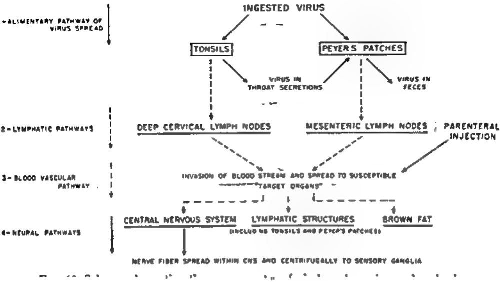
Wenner, H A, and Tanner, W A, 1948, Poliomyelitis in families attacked by the disease II The presence and appearance and persistence of neutralizing antibodies Pediatrics 2, 190-199.

Wilson, J L, 1932, Respiratory Failure in Poliomyelitis, Am J. Dis Child 43, 1433-1454

Wilson, J L, and Dickinson, D G, 1955, Prevention of long-time dependence of patients on tank respirator, J.A.M.A 158, 551-555.

Youngner, J S, 1956, Virus adsorption and plaque formation in monolayer cultures of trypsin-dispersed monkey kidney, J Immunol 76, 288-292.

SITES OF POLIOMYELITIS VIRAL MULTIPLICATION AND PATHWAYS OF VIRAL SPREAD
SHOWN SEQUENTIALLY IN CHIMPANZEES AFTER VIRUS FEEDING
(OR PARENTERAL INJECTION)



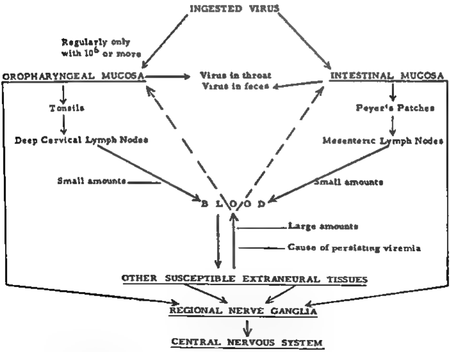


FIG 61 Schema of possible pathogenesis of poliomyelitis based on a synthesis of data available in 1956. (According to Sabin, 1956)

after feeding of avirulent strains (Sabin, 1956) Therefore, gaps in our information concerning the distribution of virus in human tissues during the earliest stages of infection can be filled tentatively by experimental data from chimpanzees sacrificed early after the feeding of virulent strains

The appearance of rising concentrations of virus has first been observed in throat secretions and in feces within 1 to 3 days after virus feeding and approximately 1 to 2 weeks prior to onset of paralysis in chimpanzees fed virulent strains The early appearance of virus in throat and feces after virus feeding thus corresponds to virus isolation at a similar interval prior to onset of symptoms in the natural infection (Gordon et al, 1947, Schabel et al., 1950) The earliest analysis of viral distribution in tissues in the presymptomatic period in chimpanzees has revealed the presence of virus only in tonsillopharyngeal tissue and in the Peyer's patches of the ileum as well as in draining lymph nodes in the deep cervical region and in the mesentery, respectively (Bodian, 1956)

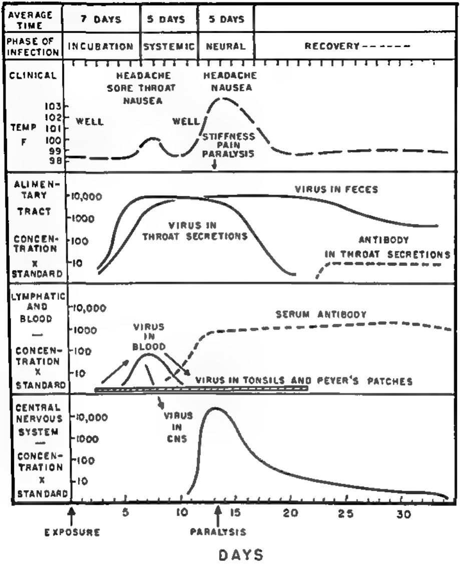
This distribution indicates that primary viral implantation and multiplication occur in one or both of two distant sites in the alimentary tract, namely, the mucosa of the throat and of the ileum Although the surface epithelia in these two sites are quite different in type, both are characterized by the presence of large aggregations of lymphatic follicles in the lamina propria of the mucosa itself. In human necropsy material, virus has been isolated with relative ease only in those tissues most frequently found infected in chimpanzees, namely, central nervous system, tonsillopharyngeal tissue, wall of the ileum, and lymph nodes (Sabin and Ward, 1941; Wenner and Rabe, 1951)

There are several points of great theoretical and practical interest in relation to the primary infection of the alimentary tract. First of all, it is probable that most infected persons, especially those exposed to avirulent strains in nature, experience only the infection in the alimentary tract and regional lymph nodes, without further spread of virus in the body. Nevertheless, such individuals may exhibit a serum antibody response and become subsequently immune to the infecting type of virus Since such individuals constitute the great majority of infected persons, they also constitute the greatest reservoir of infection for other individuals by means of the virus excreted from the alimentary tract Some of the individuals who experience no apparent viral invasion of the central nervous system, as well as many who do, suffer the symptoms of the so-called "minor illness," such as fever, sore throat and intestinal upsets. These symptoms may correspond to the primary infective process in the alimentary tract, although specific histopathologic lesions have not been clearly correlated with the presumed sites of alimentary viral multiplication Although hypertrophy of Peyer's patches has been described in autopsied cases, these structures have not as yet been assessed separately for viral content in human material

Since the sites of viral multiplication in the alimentary tract are the obvious sources of virus which is excreted by the infected asymptomatic carrier, abortive case, or paralytic patient, it is of interest that the upper and the lower alimentary tract differ with respect to time of persistence of viral excretion. Virus recovery from tonsillopharyngeal swabs has been reported only infrequently after the first week following onset of the major illness, whereas virus recovery from feces is readily accomplished during the first 3 weeks after onset Recovery from feces is often possible for several weeks or even for months after onset

LYMPHATIC AND HEMATOGENOUS VIRAL DISSEMINATION

There is no doubt at the present time that shortly after the primary stage of infection in the wall of the alimentary tract, virus is shed inwardly into lymphatic and blood vascular channels as well as outwardly into the alimentary lumen The exact mechanism of the humoral shedding is unknown, but it results in the spread of virus to regional lymph nodes, to the presence of appreciable amounts of virus in the blood serum early in the period before onset of the major illness (Horstmann et al, 1954, Bodian and Paffenbarger, 1954), and to the finding of virus in systemic lymph nodes, such as axillary and inguinal



infect 50 per cent of test units (monkeys, mice, or tissue cultures). The "standard"

nodes, in autopsied cases (Wenner and Rabe, 1951)

Whereas the invasion of regional lymph nodes from the alimentary mucosa has little or no clinical importance and may lead only to reinforcement of the immune response which probably initially occurs in the lymphoid tissue of the mucosa, the invasion of the blood stream by virus is fraught with more ominous possibilities The source of the virus which enters the blood stream is unknown, although it is suspected that virus from the lymph nodes may contribute to the viremia After initial stages of viremia in chimpanzees, the dissemination of virus to systemic lymph nodes and the brown fat of suprasternal, upper axillary, and paravertebral regions may lead to secondary sites of viral multiplication, particularly in the brown fat In this tissue virus has been found in extremely high concentration in experimental primates (Shwartzman et al , 1955, Bodian, 1956), and this leads to the possibility that reinforcement of later stages of viremia may occur from virus shed from the brown fat

The occurrence of a stage of viremia analagous to that which occurs in other viral infections in the presymptomatic period also raises the question of whether invasion of the central nervous system occurs by way of the blood stream, just as invasion of the target organs of the arthropod-borne viruses and viruses of the exanthems and of hepatitis occurs by way of this route Although the occurrence of viremic invasion of the central nervous system in the human infection is held by some to be of importance, controversy on this issue still continues, and no final answer can yet be given Evidence for the opposing points of view is presented in detail elsewhere (Bodian, 1955 and 1956, Sabin, 1956) The importance of a viremic stage of infection and of a viremic route of central nervous system invasion by virus is 2-fold First, such a mechanism of invasion would enhance the importance of serum antibody in preventing infection of the central nervous system Second, such a mechanism has to be assessed carefully in relation to the occurrence of nonimmuno- [illegible]

rier." These factors will be considered below.

DISSEMINATION OF VIRUS ALONG NERVE FIBER PATHWAYS

Experimental work has left no doubt that poliovirus is capable of spreading along nerve fibers in both the peripheral as well as in the central nervous system (CNS). The exact mechanism of this spread is unknown. In natural infections the characteristic and restricted distribution of lesions in the CNS is so similar to that which has been shown in experimental animals to be determined by nerve fiber spread that one must assume that it also is determined by nerve fiber spread of the virus along restricted pathways in the CNS However, evidence for spread of virus along nerve fibers from the alimentary tract to the central nervous system is not available, although nerve fiber spread to the CNS following intranasal, intramuscular and intrasciatic inoculation can be demonstrated under experimental conditions in monkeys.

Figures 60 and 61 summarize two points of view concerning the present status of the modes of spread and the sites of viral multiplication in poliovirus infections

INFECTION OF THE CENTRAL NERVOUS SYSTEM

Following the relatively silent events of infection in the primary sites of multiplication in the alimentary tract, and possible intermediate sites in lymph nodes, or brown fat, an occasional infected individual experiences the effects of viral multiplication in the major secondary site, namely, the central nervous system The pathologic effects of this complex process will be considered below The time course of viral increase in nervous tissue such as the spinal cord, and of its recession, is probably similar to that in other tissues infected with certain other viral agents This time course, which is summarized in relation to other events of infection in Figure 62, is characterized by a sharp rise in viral concentration during the day prior to onset of the major illness In experimental animals where this process can be studied quantitatively, it is clear that high concentrations of virus are maintained for 1 or 2 days, following which a less precipitous but yet rapid decline of virus takes place Within 1 or 2 days the de-

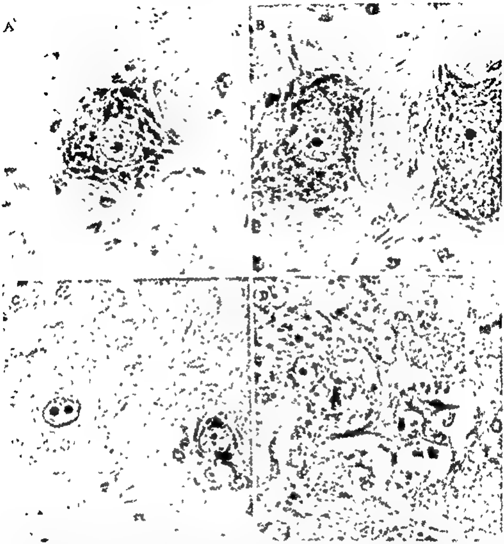


FIG 63 Regressive stages in spinal cord motoneurons in poliomyelitis early acute stage Hematoxylin-eosin-azure stain Rhesus spinal cord ×600 (A) Normal anterior *horn cell Note massive Nissl bodies in cytoplasm, central position of nucleus, large* basophilic nucleolus and dispersed chromatin (B) Early change due to virus activity Note diffuse decrease in size of Nissl bodies (chromatolysis) and tendency for aggregation of oxychromatin in nucleus (C) Severe diffuse chromatolysis, with disappearance of Nissl bodies Cytoplasm shows faint diffuse basophilia Nucleus is shrunken and contains a large eosinophilic inclusion body, as well as an intact nucleolus The inclusion body is apparently formed by clumping of oxychromatin but does not occur in all injured cells Note absence of inflammatory cells in vicinity, or of "edema" (D) Irreversibly injured motoneuron, showing neuronophagia by polymorphonuclear leukocytes and macrophages All basophilia is usually lost at this stage of necrobiosis (Photographs in Figs 63-66, 68, 70-72 by Chester F. Reather)

cline of viral concentration reaches a level which is in the neighborhood of minimal detectable virus activity by present methods of analysis. However, virus may persist at this level in experimental animals for as long as 4 weeks after onset of paralytic illness. It is not known whether the sharp damping of viral infectivity in nervous tissue is due to a generalized immune response, local immune reaction, or inhibitory factors unrelated to serum or local antibody. The decline of viral concentration is the same in animals inoculated intracerebrally and in which no appreciable serum antibody response occurs, as it is in natural infections or in experimental alimentary infections in which a high serum antibody response has already become apparent before onset of paralytic disease. Figure 62 summarizes the present state of knowledge concerning the time relations and quantitative extent of pathogenic events in relation to the clinical course of paralytic disease.

PROVOCATIVE OR PREDISPOSING FACTORS IN CENTRAL NERVOUS SYSTEM INFECTION

Experimental work of the last decade has thoroughly established the fact that strains of poliovirus may not only differ with respect to antigenic type but, within each immunologic type, exhibit striking differences in virulence or invasiveness. These differences and the ability of viral strains to invade the central nervous system from the periphery or to produce severe pathologic effects after establishing infection within the central nervous system determine to some extent the outcome of infection. In addition to these viral factors there are certain physiologic variations of the host which also play a role in determining whether an infected individual will experience

after a primary infection are (1) peripheral trauma and (2) metabolic changes in the host induced by stressful conditions. With the latter should perhaps be grouped the increased susceptibility which appears to occur during pregnancy or with increasing age in both sexes. The mechanism of action of these predisposing host factors is not clearly understood. However, there is evidence which has associated the effects of trauma and peripheral injection, as well as both recent and long-standing tonsillectomy, with an increased penetrability of the so-called "blood-brain barrier" in the portion of the central nervous system associated with the traumatized peripheral area. The more general effects of pregnancy and increased age are sometimes attributed to modifications of corticosteroid activity. For example, experimental work has clearly shown that injections of cortisone increase the risk of paralysis in animals exposed to polioviruses. The predisposing effects of peripheral trauma are also of interest in that the risk of paralysis is not only increased but the localization of initial paralysis tends to occur in the extremity which has been traumatized. The preferential localization of paralysis to a limb injected with nonviral materials such as penicillin or diphtheria-pertussis-tetanus antigen has been clearly shown to occur in epidemics, but recent evidence has confirmed the earlier impressions that the total force of this effect in any epidemic is extremely small in terms of the total number of individuals involved in the epidemic.

The mechanism of the preferential localization of paralysis to an injected limb, or to bulbar centers following recent tonsillectomy, is a matter of dispute. One point of view is that the trauma serves to localize circulating virus in the injured nerve fibers, along which ascent to the CNS occurs. Another suggested possibility is that the trauma produces a reflex increase of penetrability to circulating virus of blood vessels in the motor centers corresponding to the injured peripheral muscle.

THE NATURE OF THE LESIONS IN POLIOMYELITIS

The nature of the central nervous system lesions in poliomyelitis, as distinct from their distribution in the central nervous system, and the clinical consequences of the lesions, are 2-fold in character. The earliest, and therefore the primary, lesions are changes which appear in nerve cells in the preparalytic period. Since these changes may occur in the absence of inflammatory exudate, and in

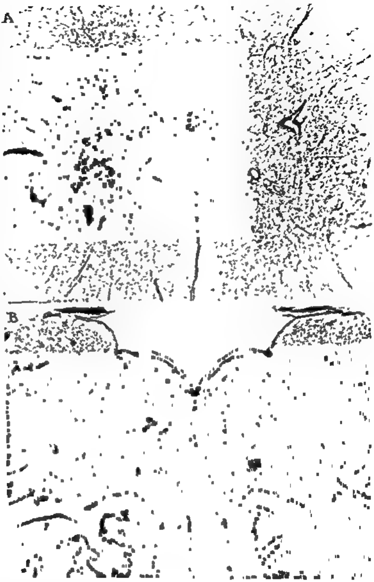


FIG. 64. Unusual human case of fatal spinal paralysis, with little brain-stem involvement H40, 17 days after onset of disease Gallocyanin stain (A) Section of lumbar cord showing complete motoneuron destruction and massive inflammatory reaction, especially on left side Note the heavy perivascular infiltrations, characteristic of the subacute and early convalescent periods (×20) (B) Same case as in A, showing the relatively slight involvement of the medulla oblongata (×10)

the margin of safety of each center before a clinical effect is observed The evidence from experimental work and from human material is overwhelmingly clear on this point Neuronal and inflammatory lesions may be found regularly in any susceptible center, including the anterior horn of the spinal cord, in persons who have never exhibited symptoms which are known to follow massive injury to such centers In experimental primates neuronal and inflammatory lesions may indeed be [illegible]

this is that severe lesions and concentrated damage to nerve cells are usually necessary *to produce* dysfunction at the clinical level, therefore, one must look for centers which are severely involved for the site of origin of clinical *signs*

experimental animals have been shown to be associated with the development of high concentrations of virus, the conclusion is inescapable that these nerve cell lesions are the *result of virus multiplication and not of the* inflammatory process which follows as a secondary response to nerve cell injury (Fig 63). Additional evidence for this view comes from the fact that in infections with some virus strains it can be shown that little inflammatory change occurs, and yet extensive nerve cell lesions and virus multiplication occur. The changes in nerve cells in the acute stage of poliomyelitis have been studied in early fatal cases in human beings and in monkeys and have been shown to be alike in the 2 species Since earlier stages and better tissue preservation can be obtained in the monkey, studies in this species have yielded more detailed information (Bodian, 1948, 1949)

Although the injury and the destruction of nerve cells may be independent of inflammatory changes, the latter do not occur in the *absence of susceptible nerve cells* For example, in regions of the thalamus deprived of nerve cells by means of retrograde degeneration following ablation of the cerebral cortex, the inflammatory changes of poliomyelitis do not occur even when virus is introduced directly into such areas This suggests that although the inflammatory reaction as it develops may be complexly determined, its origin, at least, is dependent upon a specific reaction of the virus with nerve cells

The changes in nerve cells may occur with extraordinary rapidity so that some motor nerve cells are destroyed in the preparalytic period The sequence of changes leading to destruction of cells found in the preparalytic and the early paralytic stages in rhesus monkeys is summarized in Figure 63 (A, B, C, D) A shows a normal anterior horn motoneuron B shows 2 infected motoneurons in an early stage of dissolution of cytoplasmic Nissl, bodies C shows a motoneuron with complete cytoplasmic chromatolysis, adjoining a small normal nerve cell. The chromatolysed motoneuron contains a spherical intranuclear acidophilic inclusion body, such as occurs in certain other neurotropic virus infections (Type B inclusion), but distinct from the Type A intranuclear inclusion bodies of infections with the herpes-B virus group of viruses D shows a necrotic neuron, with nucleus destroyed and cytoplasmic basophilia completely removed. Phagocytosis of this motoneuron is in progress (neuronophagia). *Many cells pass through most of these stages without evidence of surrounding inflammatory cells or exudate.* It is evident that when dissolution of the cytoplasmic Nissl substance is advanced, changes in the nucleus begin. When irreversible changes have occurred, the necrotic cell may be removed by neuronophagia due to leukocytes or macrophages or may undergo lysis

The changes in nerve cells in the acute stage are accompanied by an increase of inflammatory cells which soon may reach tremendous proportions (Fig 64A). Three principal types of such cells are found in the earliest stage: *polymorphonuclear leukocytes*, mononuclear leukocytes, and macrophages The polymorphonuclear cells may be extraordinarily numerous in some cases but persist only for a few days. The microglia are the active macrophages during the acute stage but persist in modified forms for weeks Mononuclear cells are predominantly lymphocytic cells and are present diffusely in the tissue for 2 or 3 weeks but persist as perivascular accumulations for many months.

In any attempt to analyze the relationship of lesions to clinical manifestations it is important but not necessarily easy to determine whether symptoms are the result of primary nerve cell injury or of the variable inflammatory response. These 2 processes may in some instances contribute to the same pathophysiologic result. Therefore, the clinical picture *may combine the* features of an exceedingly complex and varied disease of nerve cells and those of a variable inflammatory process in the central nervous system Added to this, one may have the baffling effect of a process *in which recoverable injury of* many nerve cells may be combined with irreversible injury to many others, in many different proportions

It is important to emphasize, moreover, *that although lesions appear in certain* functional centers in the central nervous system, symptoms attributable to such injury need not necessarily result. Such injury must reach a certain threshold of severity, varying with

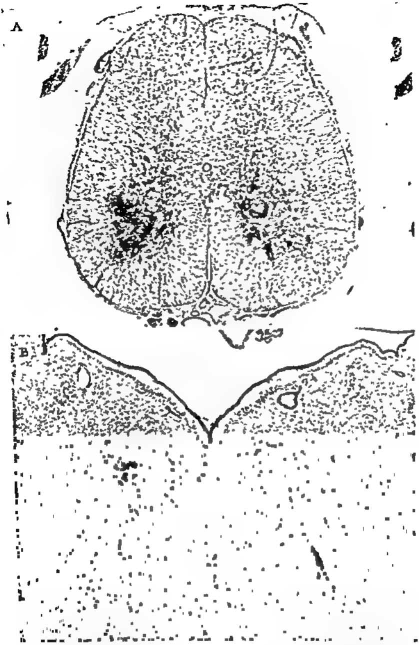


Fig. 66. Focal tissue breakdown in poliomyelitis Gallocyanin stain. (A) Small softening in lumbar cord of severely paralyzed rhesus monkey (C903), 30 days after onset of paralysis. (×20) (B) Medulla oblongata of human case, showing intense reaction in hypoglossal nuclei. On the left side the tissue has broken down in the center of the lesion Case H38, 6 days after onset of paralysis Such lesions are always small and rarely numerous (×20)

FIG 65. Photomicrographs of human spinal cords, showing that the usual association of severe inflammatory lesions with extensive nerve cell destruction does not always occur. Gallocyanin stain (×30) (A) Severe motoneuron loss in posterior part of anterior horn, with very slight inflammatory reaction Case H37, lumbar cord, 3 days after onset of illness (B) Severe focal, diffuse and perivascular infiltrations of lymphocytes and histiocytes, with most anterior horn cells relatively intact in appearance. Case H34, lumbar cord 12 days after onset of illness.

Severe inflammatory reactions in poliomyelitis are usually but not always associated with extensive nerve cell destruction (Fig. 65). In some cases, especially in the brain stem, the inflammatory response in small areas may be enormous and associated at times with a small focus of tissue softening (Fig. 66). Occasionally, a small vessel in such an area may rupture, producing a petechial hemorrhage These softenings were clearly described by Harbitz and Scheel (1907) and occur only in the severest infections Such softenings are never numerous, are rarely larger than 1 or 2 mm. in size and are not the result of vascular emboli. It is not clear whether they are the result or the cause of the very dense cellular infiltration with which they are always associated It seems doubtful that nerve cell injury or destruction alone is responsible for such an unusual reaction, since

Fig. 66 Focal tissue breakdown in poliomyelitis Gallocyanin stain (A) Small softening in lumbar cord of severely paralyzed rhesus monkey (C903), 30 days after onset of paralysis (×20) (B) Medulla oblongata of human case, showing intense reaction in hypoglossal nuclei On the left side the tissue has broken down in the center of the lesion Case H38, [illegible] days after onset of paralysis Such lesions are always small and rarely numerous (×20)

in many areas of severe nerve cell destruction the inflammatory response is relatively quite mild.

We are still ignorant of those details of the cellular pathologic reaction which might explain the occasional severe inflammatory reaction Ever since the concept of the strict neurotropism of poliomyelitis virus was shaken by the successful cultivation of the virus in non-nervous tissue by Enders and his group (1949), the possibility has suggested itself that the virus, or perhaps some strains of the virus, may multiply in the supporting elements of human nervous tissue, the neuroglia, and by so doing produce more severe tissue damage than is ordinarily seen This idea, as a matter of fact, has been advanced by some neuropathologists However, even if true, it requires the difficult assumption that the neuroglia are involved only in regions of susceptible nerve cells since the distribution of lesions in poliomyelitis is the same for all virus strains and for all primate hosts. It is conceivable that an unusually high concentration of virus in small areas may produce a toxic effect apart from the ordinary pathologic effect of virus multiplication in the nerve cells At any rate, it cannot be assumed without further evidence that focal tissue breakdown is due to the multiplication of the virus in non-neuronal elements of the nervous tissue As a rule, moreover, the focal tissue breakdown is not a primary factor in producing the paralysis of poliomyelitis, since it follows the period of greatest nerve-cell damage

Another and more subtle way in which the inflammatory reaction might conceivably affect the clinically localizable functions is by temporarily interfering with synaptic transmission by nerve cells which are destined to recover from sublethal virus effects Interference with synaptic transmission by the inflammatory exudate around motoneurons might have the effect of interrupting, perhaps temporarily, the internuncial and direct reflex pathways in variable proportions

An important observation in quantitative studies of experimental poliomyelitis is that in mild cases in the first days of the disease the great majority of cells exhibit a mild degree of diffuse chromatolysis of cytoplasmic Nissl substance (Fig 63B). In the presence of slight weakness of the muscles innervated by such cells, it is clear that neurons in this state are still functional, although synaptic transmission may not be normal. Experimental evidence strongly suggests that the function of infected motor nerve cells disappears only in the stage of severe chromatolysis (Bodian, 1948). The widespread dissemination of virus among the motor nerve cell population occurs as early as the first day of paralysis Motor nerve cells which are affected either are destroyed quickly during the first few days of the disease or undergo slower recovery changes leading to complete morphologic recovery within about a month or two In limbs showing complete paralysis, recovery is, of course, rare, and in such cases it can easily be shown that only about 10 per cent, or less, of the motoneurons have survived Experimental material clearly shows that the degree of nerve-cell destruction alone can account for most of the paralysis in the subacute and early convalescent period and is also correlated with the degree of muscle atrophy In the acute stage the correlation between nerve-cell destruction and paralysis is not quite so high, so that apparently other factors also play a role in producing paralysis One of the most significant, in cases in which paralysis is not complete, appears to be the reversible injury of nerve cells. Although available human material is not adequate for this type of study, a recovery process similar to that of the monkey appears to occur in human poliomyelitis In human cases with a duration of paralytic symptoms longer than 3 days, active neuronophagia is rarely seen In most instances evidence of active destruction of nerve cells, present in earlier cases, is absent. Within a few days after onset almost the entire degenerative phase of motor nerve cells may be terminated, and the virus concentration, previously high, falls abruptly Most remaining cells after the early acute stage show a pattern of cytoplasmic Nissl substance quite different from the diffuse chromatolysis of this period The remaining Nissl substance instead tends to aggregate in heavy masses near the nerve cell membrane, leaving a pale-staining central area in the center of the cell body (Fig. 67). This appearance of central chromatolysis is characteristic of the recovery stages of nerve cells and

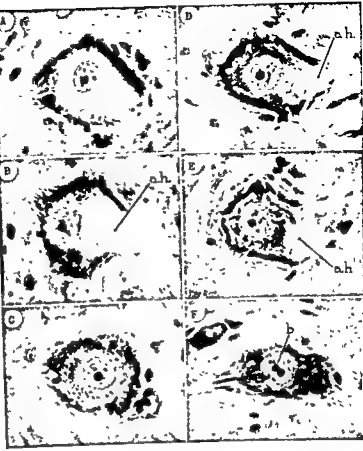


FIG 67. Rhesus B32, ninth day after onset of paralysis. (×500) (A) Severe central chromatolysis, with normal-appearing nucleus and accumulation of heavy masses of Nissl substance near cell membrane Regeneration of Nissl substance may or may not occur near the nuclear membrane. (B-E) Similar cells but with small Nissl bodies in central area. This appearance suggests regeneration of Nissl bodies from the periphery inward, with the area around the axon hillock (a h) last to show recovery (B, D and E) (F) Motoneuron of essentially normal appearance except for presence of acidophilic inclusion body (b) in nucleus In the acute stage such inclusion bodies are seen only in cells with severe chromatolysis, suggesting almost complete recovery of such a cell (Bodian, D, 1949, Poliomyelitis pathologic anatomy in Poliomyelitis. Papers and Discussions Presented at the First International Poliomyelitis Conference, pp 62-84, Philadelphia Lippincott)

has been shown by quantitative studies in monkeys to lead to complete morphologic reconstitution of most recovering cells during the subacute stage of the disease The parallel changes in nerve cells and in virus concentration in the acute and the subacute periods are summarized in Figure 68

In experimental as in human cases, anterior horn cells may be destroyed either in large groups or in scattered fashion over a period of only a few days Motor nerve fibers begin to degenerate about 3 days after destruction of their nerve cell bodies of origin and show typical morphologic changes as well as the time course of secondary or Wallerian degeneration The resulting muscle atrophy follows the time course seen, for example, in Wallerian degeneration due to nerve section, and in cases of severe paralysis is clinically visible within 1 or 2 weeks after onset of paralysis

DISTRIBUTION OF LESIONS IN THE CENTRAL NERVOUS SYSTEM

In spite of the variation in intensity of lesions which occurs in different parts of the central nervous system, partly as a result of the factors just mentioned, the over-all pattern of virus multiplication and of lesion distribution in the central nervous system is

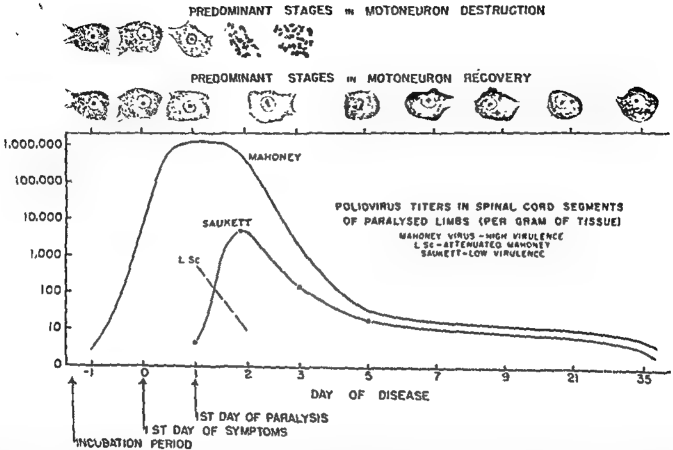
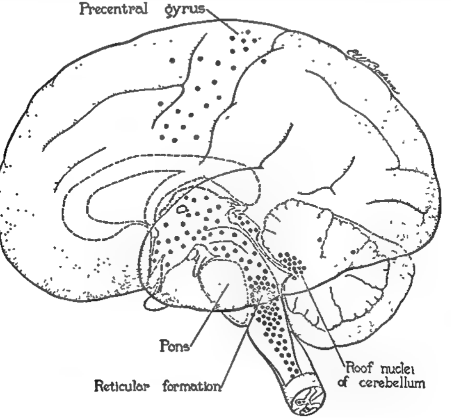
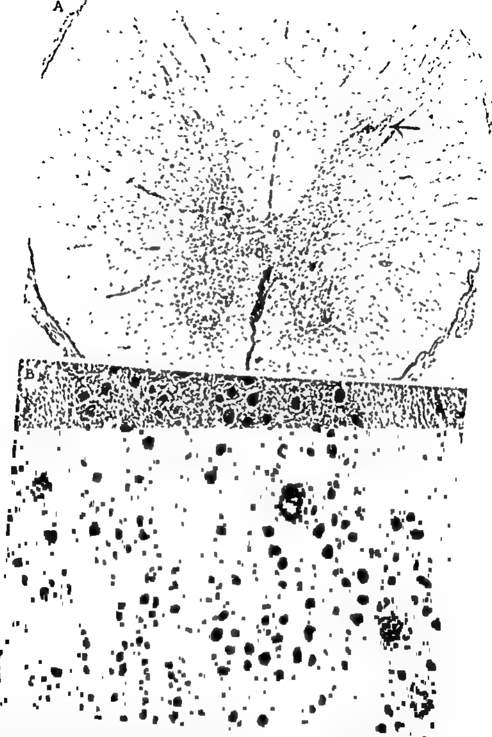


FIG. 68 Schematic representation of the sequence of the cytopathologic stages of motoneurons in the course of destruction and of those chromatolysed but able to recover. The approximate time course of changes is shown with parallel curves showing the trend of rise and decline of viral concentration in the monkey spinal cord. Note that the peak levels of viral concentration are attained at the time when the predominant stage of cell change in the motoneuron population is that of diffuse cytoplasmic chromatolysis Note particularly the low levels of infectivity of the Saukett strain on the first day of paralytic signs, even in spinal cord segments associated with severely paralyzed extremities. Mahoney and Saukett strains were tissue culture adapted strains from routine production of poliomyelitis vaccine (Eli Lilly and Company), and LSc strain was a tissue-culture adapted sample obtained by Dr. Albert B Sabin. (Bodian, D, 1958, Some physiologic aspects of poliovirus infections in The Harvey Lectures (1956-1957), Series 52, pp 23-56, New York, Acad Press)

remarkably constant. This pattern, which has been well known since the classic study of Harbitz and Scheel (1907), is quite similar in human poliomyelitis and in experimentally produced infections in monkeys and chimpanzees (Fig. 69) Thus, the clinical forms of poliomyelitis referred to as spinal, bulbar, encephalitic, meningitic and the rare cerebellar or ataxic form are due to a single pathologic picture of lesion type and lesion distribution They differ only in respect to greater severity of destructive and inflammatory lesions in one or another part of the CNS. Within the major pattern of lesion distribution, variation in details occurs In the spinal cord, for example, although some fatal cases exhibit a striking restriction of lesions to the anterior gray columns, other cases may exhibit lesions of varying severity, but usually of spotty distribution, in the intermediate, the intermediolateral and the posterior gray columns (Fig 70) Extension of virus and viral lesions to the sensory spinal ganglia is the rule, but in certain instances in which minimal lesions are present in the spinal cord, no lesions will be found in the sensory spinal



[illegible] central gyrus, and those in the cerebellum to the roof nuclei Lesions are generally found widespread in the brain-stem centers, with a number of striking exceptions, such as the nuclei of the basis pontis, and the inferior olivary nuclei (Bodian, D, 1949, Poliomyelitis pathologic anatomy *in* Poliomyelitis Papers and Discussions Presented at the First International Poliomyelitis Conference, pp 62-84, Philadelphia, Lippincott)



... may be responsible for symptoms of pain and tenderness in poliomyelitis Human case H35, 2 days after onset of disease Gallocyanin stain. (A) Cord at fifth cervical level showing inflammatory lesion in right substantia gelatinosa, as well as lesions in anterior and intermediate gray columns. (×30) (B) Eighth cervical spinal ganglion, with numerous foci of cell destruction, and surrounding inflammatory reaction. (×60)

FIG 71 Cerebral cortex and hypothalamus of a human case (H12), 9 days after onset of severe paralytic poliomyelitis Gallocyanin stain (A) Precentral gyrus (*right*), and postcentral gyrus (*left*) showing remarkable localization of lesions in precentral (motor) cortex only Lesions of greater severity are rare in fatal cases, and it is doubtful that they contribute significantly to the clinical picture (×5) (B) Hypothalamus at level of paraventricular nucleus (*below*) showing severe perivascular and focal infiltrative lesions Such lesions as well as neuronal lesions are common in fatal cases (×20)

ganglia It is important to note that some parts of the brain are rarely involved to a degree severe enough to suggest that symptoms may result The brain stem, including the hypothalamus and the thalamus, bears the brunt of the cerebral pathologic changes in poliomyelitis, lesions in the cortex, usually mild, are largely confined to the motor cortex (Fig 71) Since this is true of the encephalitic form of the disease, it is clear that the encephalitic symptoms of disorientation, restlessness, somnolence or coma must be due either to intense lesion formation in the upper brain stem, or to cortical hypoxia, rather than to the extensive cortical lesions seen in some of the other viral encephalitides In addition to the variable damage to cranial nerve centers, lesions which are highly variable in severity occur in the rostral and the caudal parts of the brain stem and in the deep cerebel-

FIG. 72. The reticular formation and vestibulocerebellar centers in human poliomyelitis Gallocyanin stain. (A) Case H32, 5 days after onset of disease Extensive nerve cell destruction and inflammatory reaction in reticular formation, vestibular nuclei (*left*), and roof nuclei of cerebellum (above IVth ventricle). Very little involvement of dentate nucleus of cerebellum (*upper right*). (×10) (B) Case H12, 9 days after onset of disease. Lesions as in (A), but especially severe in left vestibular nuclei and in right cerebellar roof nucleus (×5)

lar nuclei (Fig. 72) To these have been ascribed both motor disturbances, such as spasticity, tremor and ataxia, autonomic disturbances, including disorders of respiratory and vasomotor function, and disturbances of sweating The characteristic distribution of poliomyelitis lesions has been shown experimentally to be due to two principal factors: (1) the inherent variation of susceptibility of nervous centers to infection, and (2) the restricted movement of virus along certain nerve fiber pathways.

The distribution of lesions in the brain is essentially the same in nonparalytic cases as it is in severely symptomatic cases. This indicates clearly that symptoms are determined not only by the characteristic distribution of lesions but also by the severity of the lesions,

which varies greatly from case to case, and indeed in different parts of the nervous system of the same case. The relation of lesions in various parts of the brain to particular symptoms has been a subject of considerable interest. In considering this interesting but extremely difficult subject, one may say that variation in severity of lesions in a structure which controls so many diverse functions must necessarily lead to great variation of symptom patterns. The factors determining severity of lesions have not been studied sufficiently. Nevertheless, three general factors are known to affect this important variable. First, it is known from the earliest experimental work that virus strains differ greatly in their capacity to injure or destroy nervous tissue. Although this difference has been shown to be correlated usually with infective titer, it is not clear that this is the only determining factor. Second, it is known that severity of lesions and of symptoms may be reduced due to partial immunity resulting from previous paralytic or nonparalytic infection. Third, experimental work has shown clearly that individual hosts vary considerably in their response to a similar infective dose, even when they have had no previous immunizing experience with the virus. In view of the heterogeneity of the human population with regard to age, sex, nutritional status, endocrinologic make-up and genetic constitution, it is not surprising that all of these factors at one time or another have been explored to explain the variation of host-response. In addition, provoking factors such as trauma, strenuous exercise and certain types of immunizing inoculations must be considered as part of this complex and elusive problem.

DISTRIBUTION OF LESIONS IN RELATION TO DIAGNOSIS

Although the pathologic diagnosis of poliomyelitis is now usually a confirmatory procedure in relation to a typical clinical course, or to positive virologic and serologic findings, it may be of decisive importance in relation to atypical cases, such as instances of death in adults following acute laryngeal paralysis unaccompanied by other telltale symptoms. In such cases, fatal instances of the Guillain-Barre syndrome, or instances of fatal encephalomyelitis in which virus cannot be isolated, the burden of diagnosis leans heavily on the resources of the pathologist.

Often the spinal cord sections alone will serve to distinguish clearly between the possibilities of poliomyelitis and demyelinating disease, Guillain-Barre syndrome, or other neurologic diseases. The differentiation between poliomyelitis and other neurotropic virus diseases may further require analysis of the distribution of lesions in the brain, since spinal cord lesions are similar in many respects in most of these diseases. For this purpose multiple tissue blocks are necessary from the major functional centers of the brain.

The terminal distribution of lesions in the brain, which coincides in general with the virus distribution (Sabin and Ward, 1941), is that of a brain-stem encephalitis. Lesions in the forebrain are confined almost exclusively to the precentral gyrus, or motor cortex (Fig. 71), and the globus pallidus. Occasional cuffed vessels may be found in cortex closely adjoining the motor cortex. The olfactory, visual and auditory centers in the forebrain as well as the hippocampal formation are entirely spared.

The hemispheres of the cerebellum and the corticopontine system from cerebral cortex to pontine nuclei are free of lesions, whereas the cerebellar vermis and the deep cerebellar nuclei are often severely involved. This involvement is associated with heavy lesions in the vestibular nuclei (Fig. 72). Other centers involved in the hindbrain are the reticular formation throughout its extent and motor and sensory nuclei of the cranial nerves. In bulbar poliomyelitis unusually severe lesions are often found symmetrically placed in the region of the nucleus ambiguous on both sides of the reticular formation. Further details may be found by consulting earlier accounts (Bodian, 1947, 1949).

The characteristic distribution of CNS lesions in poliomyelitis infections is also of value in certain aspects of viral diagnosis in experimental primates. For example, in connection with the monkey safety test for poliomyelitis vaccine, the pathologic picture serves to distinguish between CNS infection due to polioviruses and silent infections due

to viruses of simian origin (Technical Committee, 1956).

REFERENCES

Bodian, D, 1947, Poliomyelitis, neuropathologic observations in relation to motor symptoms, *J A M A* *134*, 1148-1154

———, 1948, The virus, the nerve cell, and paralysis, a study of experimental poliomyelitis in the spinal cord, Bull Johns Hopkins Hosp *83*, 1-107

———, *1949, Poliomyelitis* pathologic anatomy *in* Poliomyelitis [illegible] the First [illegible] pp 62-84, [illegible]

———, 1952, [illegible] of poliomyelitis, Am J Hyg *55*, 414-438

———, 1955, Emerging concept of poliomyelitis infection, Science *122*, 105-108

———, 1956, Poliovirus in chimpanzee tissues after virus feeding, Am J Hyg *64*, 181-197

———, 1957, Mechanisms of infection with polioviruses *in* Cellular Biology, Nucleic Acids and Viruses, pp 57-73, Special publication, New York Academy of Sciences

———, 1958, Some physiologic aspects of poliovirus infections *in* The Harvey Lectures (1956-1957), Series *52*, pp 23-56, New York, Acad Press

Bodian, D, and Paffenbarger, R S, Jr, 1954, Poliomyelitis infection in households, frequency of viremia and specific antibody response, Am J Hyg *60*, 83-98

Enders, J F, Weller, T H, and Robbins, F C, 1949, Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues, Science *109*, 85-87

Gordon, F B, Schabel, F. M, Casey, A E, and Fishbein, W. I, 1947, Recovery of poliomyelitis virus from the throat during the incubation period, *J A M A* *135*, 884-888

Harbitz, F., and Scheel, O, 1907, Akute Poliomyelitis und verwandte Krankheiten Pathologisch-anatomische Untersuchungen aus den Epidemien in Norwegen, 1903-1906 Christiana, Dybwad (Also in J A M A *49*, 1420)

Horstmann, D M, McCollum, R W, and Mascola A D, 1954, Viremia in human poliomyelitis, J Exper. Med *99*, 355-369

Sabin, A B, 1956, Pathogenesis of poliomyelitis Reappraisal in the light of new data Science *123*, *1151-1157*

Sabin, A B, and Ward, R, 1941, The natural history of human poliomyelitis I Distribution of virus in nervous and nonnervous tissues J Exper Med. *73*, 771-793

Schabel, F M, Smith, H. T, Fishbein, W I, and Casey, A E (with the cooperation of Bundesen, H N) 1950, Stool virus recovery in subclinical poliomyelitis during incubation, febrile, and convalescent periods J Infect Dis *86*, 214-218

Shwartzman, G., Aronson, S M, Teodoru, C. V, Adler, M, and Jahiel, R, 1955, Endocrinological aspects of pathogenesis of experimental poliomyelitis, Ann New York Acad Sc *61*, 869-876

Technical Committee on Poliomyelitis Vaccine and Subcommittee on the Monkey Safety Test, 1956, The monkey safety test for poliomyelitis vaccine, Am J Hyg *64*, 104-137.

Wenner, H A., and Rabe, E F., 1951, The recovery of virus from regional lymph nodes of fatal human cases of poliomyelitis, Am J M Sc *222*, 292-299.

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24

Poliomyelitis: Control

INTRODUCTION

Although the report (Landsteiner and Levaditi, 1909) that the virus of poliomyelitis was transmitted to an experimental animal was made in 1909, it was not until 1931 that suspicion was expressed concerning the possible existence of more than one immunologic variety (Burnet and Macnamara, 1931). This was soon confirmed by many workers, and in 1949 the differentiation of 3 immunologic types was clearly established (Bodian et al, 1949, Kessel and Pait, 1949). On the basis of findings in a study of 100 strains (National Foundation for Infantile Paralysis, Committee on Typing, 1951), it seemed probable that the development of a procedure for the control of poliomyelitis by immunologic means need be concerned only with the 3 immunologic varieties of virus then known to cause the paralytic disease. More recent evidence suggests that sporadic cases, as well as small epidemics of the poliomyelitis syndrome, including paralysis, are caused by such immunologically diverse agents as Coxsackie and ECHO viruses (see Chaps 25 and 26). Among the 3 immunologic varieties of poliovirus, type I is the principal contributor to the paralytic form of the disease, while all 3 types seem to cause infection equally readily (Salk, 1955a).

Lifelong immunity to paralysis results from natural infection (Paul and Riordan, 1950), and it seems unnecessary to postulate that the maintenance of immunity is due to repeated reinfections. When infantile infection occurs, under the protection of maternal antibody, sustained immunity to paralysis results, but not necessarily immunity to reinfection.

Among the earliest reports on immunization of monkeys, and then of man, were those of Brodie and Park (1936) and Kolmer (1936). Their studies were conducted before precise knowledge of immunologic complexity was available. More than a decade later, Morgan (1948) clearly demonstrated that a formaldehyde-treated suspension of central nervous system tissue from monkeys containing a type II strain of poliovirus caused the formation of appreciable quantities of antibody. She was also able to induce a measurable degree of resistance to intracerebral challenge in monkeys vaccinated with similarly prepared type I virus. A number of other investigators (Loring et al, 1947, Milzer et al, 1945, Schwerdt et al, 1951) in studies with mice and cotton rats, confirmed the fact that preparations of type II virus that were noninfectious were still capable of inducing immunity in rodents. These studies indicated that materials devoid of demonstrable infectious activity for animals will immunize if given repeatedly in sufficient amount.

This approach was extended by Howe (1952) who was able to demonstrate antibody formation in chimpanzees. In parallel experiments in 6 human subjects he observed the development of antibody that in most in-

stances persisted over the observation period of 6 months.

The discovery by Enders et al. (1949) of the cultivation of poliovirus in various human embryonic tissues, and the independent observation of Smith et al (1950) of the growth of polioviruses in culture of human testicular tissue, initiated and stimulated studies that led to the demonstration in monkeys and then in man (Salk et al 1953) of the antigenic activity of formalin-treated virus grown in tissue culture, and the later demonstration (Francis et al, 1957) of the protective effect of formalinized vaccine (Salk, 1955b) prepared from virus propagated in cultures of monkey kidney tissue

In another approach for inducing immunity, first tested in humans by Koprowski et al (*1952*), they reported the results of studies in 20 human subjects fed an attenuated live type II virus which had been propagated in the CNS of rodents Evidence was found of virus multiplication in the gastro-intestinal tract, and an immunologic response occurred The agent used for feeding was less pathogenic for the monkey than for rodents, but still caused paralysis when inoculated intracerebrally into the monkey Studies with virus that had undergone greater degrees of attenuation have been further extended by Koprowski (1957) and by Sabin (1957)

A fuller historical account of studies on immunology and immunization against poliomyelitis is presented by Boyd (1953, 1957), and the reader is referred to these well-documented sources The following sections deal with the substance of recent studies that bear directly upon the control of poliomyelitis by immunologic means.

Passive Immunization

That it is possible to induce passive immunity artificially, simulating the effect of passively transferred maternal antibody, was demonstrated not only in experimental animals (Bodian, 1951) but also in human subjects given large doses of human gamma globulin (Hammon et al, 1953) Although a clearly demonstrable effect was induced in a carefully controlled field trial and revealed that a low level of antibody was effective in prevention of paralytic polio, the procedure proved to be impractical for general use because of the large amounts of gamma globulin required for so many potential susceptibles who might not be exposed, and the relatively short period of duration of gradually diminishing effect in relation to the uncertainty of time of exposure.

Active Immunization

That it should be possible to induce lifelong immunity to paralysis by artificial means is suggested by the fact that immunity of this degree develops under natural circumstances (Paul and Riordan, 1950). On the basis of known immunologic principles and experience, two approaches could be considered: (1) the use of orally administered attenuated virus for inducing infection and simulating the natural immunizing process, and (2) the use of a noninfectious virus preparation for inducing the formation of circulating antibody and, [illegible]

of considerable discussion, both theoretical and practical, and because all issues have not been fully resolved and are broadly related to the problem of the control, not only of poliomyelitis but also of other virus diseases, each will be considered in detail

Before doing so it might be well to point out certain conceptions that have prevailed which have influenced thought and the direction of experimentation. It is a fact that the [illegible]

low fever). It does not necessarily follow, [illegible]

the effective immunologic control of any virus disease be brought about The evidence suggesting that immunity induced by killed-virus vaccines is sometimes less substantial and less durable than that resulting from infection does not necessarily mean that the critical elements for solid and durable immunity cannot be created by a noninfectious preparation suitably prepared and suitably administered

LIVE-VIRUS VACCINE

Prerequisites

The prerequisites for a live-virus vaccine for poliomyelitis are: (1) *biologic attenuation* of virulence before human use, and (2)

knowledge of the degree of stability of the attenuated form when released in the human population It is self-evident that the application of such a procedure must be without risk to the treated individual, to his immediate contacts and to the community beyond

A very considerable body of experimental data has been developed by Koprowski (1957) and by Sabin (1957) and by others on attenuation of virulence by various biologic devices for segregation and propagation of relatively avirulent virus populations The reader is referred to the work of these investigators for a full account of the technics employed and the results obtained in effecting a reduction in virulence for the CNS of laboratory primates Although there is not full agreement upon the degree of attenuation to be achieved before application of attenuated polioviruses in experiments involving increasing numbers of human subjects, the evidence deduced by Koprowski and Sabin, and others, in several hundred persons over a period of several years, has revealed no harmful effect However, a return of virulence for primate CNS was shown in tests of the excreted virus, in a number of instances at least (Sabin, 1957, Dane et al, 1957, Dick et al, 1957, Dick and Dane, 1957) Moreover, it was shown that virus spread to contacts (Dane et al, 1957, Dick et al, 1957, Dick and Dane, 1957; Paul et al, 1957) Some of these studies have also shown that a degree of attenuation could be achieved that would result in retention of infectivity for tissue culture, but reduced capacity to multiply in the human alimentary tract (Sabin, 1957) Such strains also failed to immunize Thus, retention of power to infect is accompanied by the power to multiply and to spread to contacts and has been associated with the possibility of reappearance of forms virulent for the CNS of laboratory primates (Sabin, 1957) The significance of these findings, particularly in relation to the question of the limitations within which such preparations are safe for the individual or the community, cannot possibly be resolved without direct test in large numbers and under circumstances that would avoid possible confusion of results by the activity of naturally occurring polioviruses It would be necessary to evaluate the effects of intangible factors of time and season, as these bear on the appearance of virulent forms selected from the naturally predominantly avirulent forms, and the phenomenon of mutation of the artificially attenuated viruses

The problem posed by the possible use in man of a live-virus vaccine for poliomyelitis is somewhat different from that of a live-virus vaccine for smallpox or yellow fever In the instance of attenuated yellow fever virus, there is less risk of person-to-person transmission, without a mosquito vector, and the duration of virus carriage in the inoculated host is sharply limited. This is quite different for poliovirus in man where both naturally occurring and artificially administered viruses have been shown to be present, in many instances for many weeks, in the human gastrointestinal tract In the case of smallpox, it appears that vaccinia virus, which has limited capacity to spread (except in such instances as persons with eczema) and limited persistence in the vaccinated individual, has stable pathogenic properties

In view of the demonstration that it is possible to induce immunity to paralytic poliomyelitis by means of a killed-virus vaccine, and in view of the implications of the foregoing, some have questioned the reasonableness of the live-virus vaccine approach The principal incentive for continued interest in the possible usefulness of a live-virus vaccine (Paul et al, 1957) resides in the unresolved question concerning the duration of immunity that it is possible to achieve with a killed-virus preparation Additional reasons are related to ease of oral administration and possible economy of material if smaller amounts of live-virus can be employed than are required for injection of killed antigen It has been proposed that the feeding of attenuated viruses might ultimately exclude virulent virus from circulation (Koprowski, 1957, Fox, 1957).

It is difficult to follow the reasoning that an attenuated live virus might exclude virulent viruses from circulation in the population inasmuch as the basis for the relationship between virulent and avirulent forms in nature is not known It is also difficult to understand how the eradication of poliovirus infection from a population would be effected by the use of a live-virus vaccine when this has

not occurred under circumstances of a high immunity index in areas of poor hygiene and high infection rate but low paralytic rate. Since the reservoir of infection would be maintained artificially by inducing infection in the newborn, it is to be expected that the paralytic rate in the population would be less than in the absence of the application of some method of artificial immunization early in life

There is no reason to question the probable high order of effectiveness of the immunizing potential of a live-virus vaccine. Some have expressed the view that if all persons throughout the world could be fed the same type of attenuated, live-poliovirus on the same day, the question of possible risk to contacts would be obviated, and the risk of future newborn could be obviated by early feeding of live-virus while maternal antibody is still present in protective amounts

Since this has not yet been accomplished, and since it is highly improbable that this could be done, then the questions posed concerning the practicability of a live-virus vaccine, for a disease with the characteristics of poliomyelitis, still remain to be answered.

KILLED-VIRUS VACCINE

Prerequisites

The essential prerequisites for an effective immunizing procedure, using a killed-virus vaccine, are: (1) a practicable source of virus for conversion into vaccine; (2) a reliable method for destroying infectivity while retaining antigenicity; and (3) a practicable way for inducing an effective degree of immunity that is sufficiently long-lasting

Source of Virus

The demonstration by Enders, Weller and Robbins (Enders et al, 1949) that poliomyelitis viruses can be propagated in a variety of human and simian tissues was a key factor in providing the basis for a source of virus for vaccine, and for the development of simplified technics for measuring both virus and antibody activity. The availability of antibiotics provided the basis for overcoming one of the principal obstacles to mass tissue cultivation and in vitro testing The finding that monkey kidney tissue cultures provided a rich source of virus permitted the rapid extension of early observations that virus grown in tissue culture could be rendered noninfectious and still retain antigenic power (Salk et al., 1953).

It is probable that continuously propagating cell-lines will ultimately be used as a source of virus for poliomyelitis vaccines and will eliminate dependence on monkeys for the cell substrate upon which virus for vaccine is propagated

Reliable Method of Killing

The conversion of the live-virus to a noninfectious form can be effected by treatment with formaldehyde. In the presence of a 1:4,000 dilution of formalin at pH 7 and at 37° C. the reaction is essentially complete in less than 1 week; but, in general, from 3 to 5 days of additional treatment is applied. The killing process is readily completed, with an adequate margin of safety, if the raw tissue culture fluid is first clarified by filtration for removal of particles that might contain entrapped virus that could not make effective contact with formaldehyde (Salk et al, 1954 and 1955; Salk, 1956b)

The indication that the inactivation of polioviruses, by formaldehyde, is an orderly and predictable phenomenon suggested that the inactivation procedure could be amenable to the kind of control that would permit reliably reproducible destruction of infectivity, with retention of antigenicity (Fig 73).

The importance of understanding the na-
ture of the [illegible]

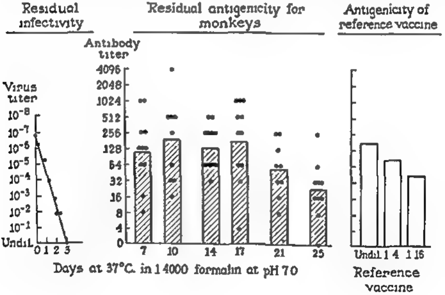
infectious virus exists in a vaccine preparation is so high as to provide practicable assurance that complete inactivation has, in fact, taken place In practice, the controls for vaccine preparation are as follows: Samples are removed at intervals to reveal the rate of loss of infectivity with time; residual virus infectivity is plotted on a logarithmic scale, and time on an arithmetic scale This is done to indicate that a sufficient amount of free-formaldehyde is present in the particular reaction vessel to cause the inactivation to proceed in the expected manner. Overtreatment is then applied, for a sufficient margin of safety, to increase the probability that the entire contents of the vessel are rendered noninfectious. Samples for safety test are removed after the process is completed. If the results

of tests upon such samples are negative, and if this occurs consistently in consecutive lots, this indicates that a reproducible method is available, and it provides a basis for reasonable expectation that each dose of each lot, so prepared and tested, is equally free of infectious virus

In the event that the measurable portion of the line describing the course of the inactivation reaction proceeds as expected, and if, in relation to the data on rate of inactivation, the safety test samples are positive when theoretically they should be negative, a clue is provided that something is awry (Salk, 1956b). Wherever such difficulties have been encountered in failure to inactivate virus consistently, it has been found that this can be rectified by the use of a suitable system of clarification and filtration for removal of particles that contain virus protected from contact with formaldehyde (Bodian et al, 1955, Salk, 1956b, Salk, 1957a) It is now well established that in practice, as well as in theory, safe vaccine can be prepared consistently (Bodian et al, 1955; Bodian, 1958).

It was reported originally that inactivation of poliovirus proceeds as does a first-order reaction (Salk et al, 1954; Salk et al, 1955) There is considerable evidence suggesting that the inactivation is essentially first-order (Salk, 1957a, Bodian, 1958) as has been demonstrated in a number of laboratories However, the experience of others indicates deviations from a straight line at either the beginning (Timm et al, 1956) or at the end (Gard and Lycke, 1957) of the measurable portion of the inactivation curve These differing experiences have not yet been reconciled.

Attempts have been made to use ultraviolet light for destroying infectivity of poliovirus for vaccine, however, too great loss in antigenicity occurs after sufficient treatment for destruction of infectivity Ultraviolet light has been used in conjunction with formalde-



of Reference Vaccine A These data are for the Saukett strain of type III virus (Salk, J E., 1957, Viral and cellular factors pertinent to control of paralytic poliomyelitis with a noninfectious vaccine *in* Cellular Biology, Nucleic Acid and Viruses (Special Publication), vol. 5, pp 77-89, New York Acad Sc.)

not occurred under circumstances of a high immunity index in areas of poor hygiene and high infection rate but low paralytic rate. Since the reservoir of infection would be maintained artificially by inducing infection in the newborn, it is to be expected that the paralytic rate in the population would be less than in the absence of the application of some method of artificial immunization early in life

There is no reason to question the probable high order of effectiveness of the immunizing potential of a live-virus vaccine. Some have expressed the view that if all persons throughout the world could be fed the same type of attenuated, live-poliovirus on the same day, the question of possible risk to contacts would be obviated, and the risk of future newborn could be obviated by early feeding of live-virus while maternal antibody is still present in protective amounts

Since this has not yet been accomplished, and since it is highly improbable that this could be done, then the questions posed concerning the practicability of a live-virus vaccine, for a disease with the characteristics of poliomyelitis, still remain to be answered

KILLED-VIRUS VACCINE

Prerequisites

The essential prerequisites for an effective immunizing procedure, using a killed-virus vaccine, are. (1) a practicable source of virus for conversion into vaccine; (2) a reliable method for destroying infectivity while retaining antigenicity, and (3) a practicable way for inducing an effective degree of immunity that is sufficiently long-lasting.

Source of Virus

The demonstration by Enders, Weller and Robbins (Enders et al, 1949) that poliomyelitis viruses can be propagated in a variety of human and simian tissues was a key factor in providing the basis for a source of virus for vaccine, and for the development of simplified technics for measuring both virus and antibody activity. The availability of antibiotics provided the basis for overcoming one of the principal obstacles to mass tissue cultivation and in vitro testing The finding that monkey kidney tissue cultures provided a rich source of virus permitted the rapid extension of early observations that virus grown in tissue culture could be rendered noninfectious and still retain antigenic power (Salk et al, 1953).

It is probable that continuously propagating cell-lines will ultimately be used as a source of virus for poliomyelitis vaccines and will eliminate dependence on monkeys for the cell substrate upon which virus for vaccine is propagated.

Reliable Method of Killing

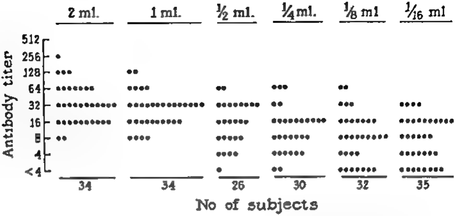
The conversion of the live-virus to a noninfectious form can be effected by treatment with formaldehyde. In the presence of a 1:4,000 dilution of formalin at pH 7 and at 37° C. the reaction is essentially complete in less than 1 week; but, in general, from 3 to 5 days of additional treatment is applied The killing process is readily completed, with an adequate margin of safety, if the raw tissue culture fluid is first clarified by filtration for removal of particles that might contain entrapped virus that could not make effective contact with formaldehyde (Salk et al, 1954 and 1955, Salk, 1956b)

The indication that the inactivation of polioviruses, by formaldehyde, is an orderly and predictable phenomenon suggested that the inactivation procedure could be amenable to the kind of control that would permit reliably reproducible destruction of infectivity, with retention of antigenicity (Fig 73).

The importance of understanding the nature of the inactivation reaction, for establish- [illegible] of safety is to provide

infectious virus exists in a vaccine preparation is so high as to provide practicable assurance that complete inactivation has, in fact, [illegible]

time on an arithmetic scale. This is done to indicate that a sufficient amount of free-formaldehyde is present in the particular reaction vessel to cause the inactivation to proceed in the expected manner. Overtreatment is then applied, for a sufficient margin of safety, to increase the probability that the entire contents of the vessel are rendered noninfectious Samples for safety test are removed after the process is completed If the results



[illegible] antibody to any type prior to Reference Vaccine A given [illegible] The fractional amounts of [illegible] ml injection. (Salk, J E, 1955, Considerations in the preparation and use of poliomyelitis virus vaccine, J A M.A. *158*, 1239-1248)

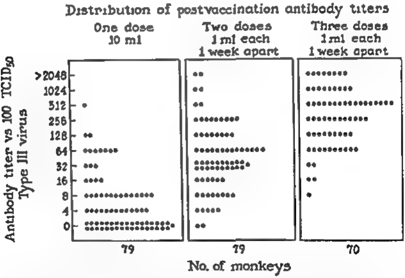


FIG 76 Comparison of antibody response to multiple doses and a single large dose as tested in monkeys Each symbol represents the antibody level for type III virus in 1 monkey injected either with 1 10-ml dose or 2 doses of 1 ml each a week apart or 3 doses of 1 ml. each a week apart (Salk, J E, 1955, Studies with noninfectious poliomyelitis virus vaccines *in* Poliomyelitis: Papers and Discussions Presented at the Third International Poliomyelitis Conference, pp 167-185, Philadelphia, Lippincott)

hyde (Taylor et al., 1957), but there is no evidence that this is either necessary or advantageous

Factors of Importance for Inducing an Antigenic Effect with a Noninfectious Vaccine

It is possible to destroy infectivity completely, with full retention of antigenic activity (Salk, 1957a) Loss of antigenic substance in the course of vaccine manufacture and processing can occur through removal of antigen by filtration, or through excessive destruction by the chemical or physical factors employed for destroying infectivity, or by the use of certain preservatives of sterility that deleteriously affect antigen stability. The degree of antibody response elicited in experimental animals and in man is related to the 3 fundamental factors: (1) potency of the antigen administered, (2) number of injections and (3) spacing between injections. These and other factors that influence the formation and the persistence of antibody will be discussed in relation to the question of the mechanism of immunity and the control of the disease.

Normal Biologic Variation

In considering the influence of quantity of antigen upon degree of antibody formation, cognizance must be taken of the relatively wide degree of variation in individual response not only among human subjects but in experimental animals as well This is only another example of normal biologic variation and emphasizes the expectation that some individuals will react with antibody formation to the injection of small quantities of antigen whereas other individuals will not respond. The basic factors responsible for the absence of uniformity in responsiveness is beyond the scope of the present considerations However, the fact itself is of fundamental importance because the degree of effectiveness of any procedure employed for artificial immunization must consider the extremes of variation likely to be encountered.

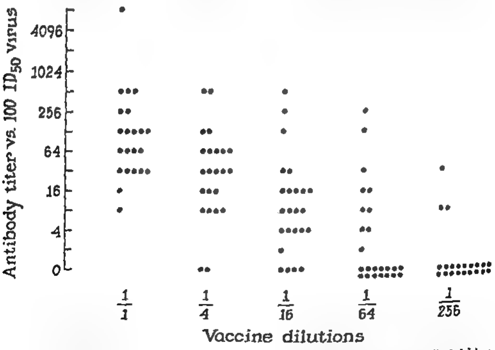


FIG. 74. Antibody response in groups of monkeys vaccinated with diminishing dilutions of Reference Vaccine A. Three doses were given intramuscularly at weekly ... third dose. Each symbol represents ...ent of Reference Vaccine A. (Salk, ...use of poliomyelitis virus vaccine,

ployed was occurring as the interval between injections was prolonged (Salk, 1955b).

Factors Related to Practical Application

The translation of these observations into practical application must consider the length of the interval between the first injection and the time when it is desired that a satisfactory degree of immunity is engendered (Salk, 1956c and 1957b) It would seem from the foregoing that, within certain limits, any desired effect could be achieved by adjusting vaccine potency, number of injections and spacing between injections For example, if a 1-dose procedure were desired, a greater potency of vaccine would be required to induce the formation of a selected mean level of antibody, or conversion of a certain proportion of persons from negative to positive antibody status, than would be required to achieve the same level of antibody response if vaccine is given on a 2- or a 3-dose schedule Furthermore, the requirements for potency would be less if intervals between injections are longer rather than shorter. The greatest assurance of maximal effect would be provided by vaccine at a level of potency that would be expected to effect conversion from negative to positive antibody status (i e, titer of 1:4 or > vs 100 ID_{50} of virus) in almost all persons after 2 doses of vaccine, separated by an interval of 1 month, allowing a third dose, some months later, to provide a further margin for full effectiveness, as well as the booster effect.

Effect of Intensity of Primary Sensitization upon Degree of Response to Booster

Another factor that determines the level of antibody that can be induced by a killed-virus vaccine is the degree of primary antigenic stimulation Figure 79 reveals the response to a fixed dose of vaccine administered as a booster injection in groups of individuals treated earlier with graded doses of Reference Vaccine A It is evident that the response to the booster dose is influenced not only by the size of this dose (Salk, 1955b)

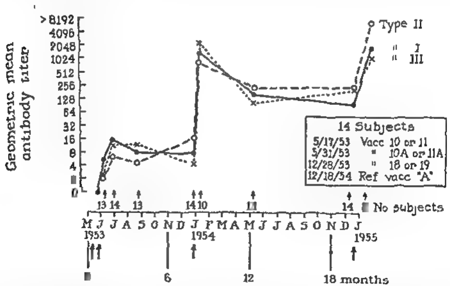


Fig 78 Illustration of "booster" response to third dose given approximately 7 months after 2 primary injections spaced 2 weeks apart Geometric mean antibody titers in 14 children who possessed no discernible antibody prior to injection. Degree of persistence after third dose and response to a fourth dose administered 1 year later are illustrated also Number of subjects available for serologic study at each point in time is shown above the time scale (Salk, J E, 1955, Considerations in the preparation and use of poliomyelitis virus vaccine, J A M A. *158, 1239-1248*)

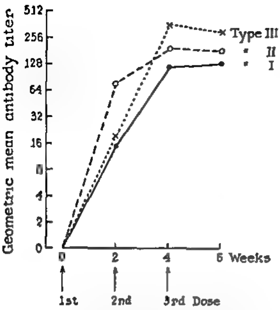


FIG 77. Refractoriness to further increase in antibody titer after third injection of series of 3 at 2-week intervals Each symbol represents mean for 20 individuals. (Salk, J. E. 1955. Considerations in the preparation and use of poliomyelitis virus vaccine, J.A.M.A *158*, 1239-1248)

EFFECT OF ANTIGEN DOSE

The influence of dose upon antibody response and the effect of variation in responsiveness among monkeys is shown in Figure 74 The levels of antibody observed in individual animals 1 week after the last of 3 weekly injections are shown The lack of uniform response at each dosage level is clearly evident The effect of quantity of antigen upon degree of response is shown Similar observations have been made in groups of children who had no detectable antibody to any type prior to injection This is shown in Figure 75. In this instance, 2-fold dilutions of vaccine are compared, and the response shown is that observed 2 weeks after a single 1 ml dose containing the indicated quantity of stock reference vaccine It is clearly evident that some individuals, both among monkeys and human subjects, react less readily to the same dose of vaccine than do others, and that by increasing the quantity of antigen administered, it is possible to raise the proportion of those who do respond so that above a certain minimal level of antigen it can reasonably be expected that all, or almost all, will respond—except for those who may have a defect in their immunologic mechanism

EFFECT OF SINGLE VS. DIVIDED DOSES

Data in Figure 76 are presented to illustrate the advantage of small divided doses, at intervals, as compared with a single large dose. The antibody response in monkeys, after 2 or 3 doses of vaccine of 1 ml. each, given a week apart, is compared with the level of antibody induced by 10 ml of vaccine given in a single dose.

EFFECT OF LENGTH OF INTERVAL BETWEEN INJECTIONS

The effect upon antibody response of the length of the interval between injections is illustrated by a comparison of Figures 77 and 78 Figure 78 reveals that 3 doses in man was not as efficient, when given over a 4-week period, as was spacing extended over a 7-month period. There is a period of relative refractoriness to further antibody response early after primary immunization, this is then followed by the development of a markedly hyper-reactive state. The length of the interval required for the development of the hyper-reactive state differs in different animals. The interval required in man is longer than in the monkey; and in this animal it is longer, in turn, than in small laboratory animals such as guinea pigs and mice Although the precise reasons for these differences are not clear, they may well be linked to the rate of metabolism and rate of maturation in the respective species. Although systematic studies are incomplete, the results in man suggest the advantage of longer rather than shorter intervals; and, in 4 to 5 months, after a single injection (Salk, *1955b*), some degree of hyper-reactivity is evident in most individuals It is probable that the point in time when the hyper-reactive state is developed varies from person to person Since it is not possible to study this phenomenon in a single individual, the result of such a study in sufficiently large groups of individuals could be expected to reveal a gradual change approaching a plateau. Such observation was made in an experiment the final interpretation of which is limited by the fact that some degree of deterioration of the vaccine em-

Group A, but a lesser proportion of individuals had demonstrable antibody 1 year later. The effects observed for the types II and III component of the vaccine were more substantial, and the degree of persistence was greater The conclusion to be drawn is that the level and the degree of persistence of antibody after the booster is related, in part, to the intensity of antigenic stimulation

From the experience with the type II and type III components of the vaccines used in this study, it appears that another factor that plays a role in determining the efficiency of antibody formation and persistence is the quality of the antigen of the particular strain of virus used for conversion into vaccine. It is generally true that different antigenic substances possess different degrees of antigenic capacity—some are good antigens, and others are inferior. However, the same quantitative factors play a role in determining the response that can be achieved within the limits of capacity of any particular antigen.

The course of antibody rise and decline is illustrated in Figure 81. Observations made over a 3- to 4-year period (Salk, 1958) in-

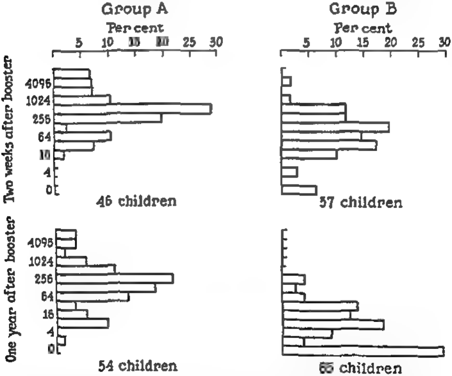


FIG 80 Distribution of type I antibody titers 2 weeks after booster injection, and again 1 year later. Comparison between 2 groups treated as follows

GROUP A Individuals given 2 doses 2 weeks apart, containing the following volumes of Reference Vaccine A. 2 ml, 1 ml or ½ ml This was followed 1 year later by "booster" dose of 1 ml. of Vaccine J essentially similar in potency to Vaccine A

GROUP B Individuals treated essentially the same way as those of Group A, except for the volumes of Reference Vaccine A used for each of the first 2 doses, which were ¼ ml, ⅛ ml. or ⅟₁₆ ml. (Salk, J E, 1958. Basic principles underlying immunization against poliomyelitis with a noninfectious vaccine *in* Poliomyelitis Papers and Discussions Presented at the Fourth International Poliomyelitis Conference, Philadelphia, Lippincott)

but also by the intensity of the primary immunologic experience. Thus, children given 2 ml. of Vaccine A for each primary injection responded to 1 ml. of Vaccine J with more antibody than did those given $\frac{1}{16}$ ml of Vaccine A for each dose of the initial treatment Figure 79 also presents, for comparison, levels of antibody induced by natural infection It illustrates that, depending upon the potency of vaccine used, the number of doses, and the length of the intervals between injections, the level of antibody induced artificially can equal or even exceed that resulting from natural infection.

Factors Affecting Degree of Persistence of Antibody

It is clear from the foregoing that any statement concerning the comparative antibody response to a killed-virus vaccine versus a live-virus vaccine would have to take cognizance of the potency of the killed-virus vaccine and the number and the spacing of injections. These factors influence not only the level of antibody induced but also the degree of antibody persistence.

Data in Figure 80 reveal that a decline in type I antibody level occurred in the course of a year following the booster dose in groups given vaccine of the degree of potency administered in Group A; however, at the end of the year, all individuals still possessed a demonstrable level of circulating antibody (Salk, 1958). A different situation is illustrated in Group B given a less potent antigen for primary stimulation, but the same dose as Group A for the booster; not only was there a lesser response as compared with

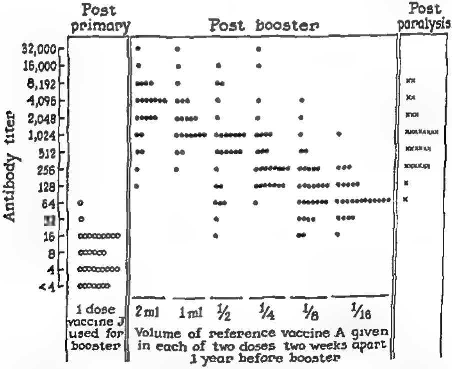
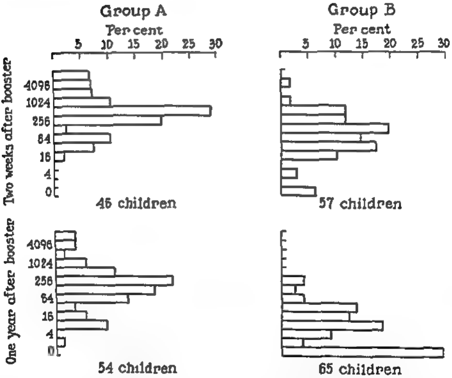


Fig. 79. Effect of amount of antigen used for primary immunization upon antibody response to "booster" dose, and comparison with antibody titer following natural infection Center portion of chart illustrates response of individuals 2 weeks after 1 dose of Vaccine J and shows the effect upon the booster response of the amount of antigenic stimulation at the time of primary immunization. The effect of 1 dose of Vaccine J (1 ml I M.) in children not previously treated with vaccine is shown in the frame on the left, and antibody levels in the convalescent phase of paralytic infections are shown in the frame on the right. (Salk, J E., 1957, Poliomyelitis vaccination in the fall of 1956, Am. J. Pub Health 47, 1-18)

Group A, but a lesser proportion of individuals had demonstrable antibody 1 year later The effects observed for the types II and III component of the vaccine were more substantial, and the degree of persistence was greater The conclusion to be drawn is that the level and the degree of persistence of antibody after the booster is related, in part, to the intensity of antigenic stimulation

antibody formation and persistence is the quality of the antigen of the particular strain of virus used for conversion into vaccine It is generally true that different antigenic substances possess different degrees of antigenic capacity—some are good antigens, and others are inferior. However, the same quantitative factors play a role in determining the response that can be achieved within the limits of capacity of any particular antigen

The course of antibody rise and decline is illustrated in Figure 81 Observations made over a 3- to 4-year period (Salk, 1958) in-



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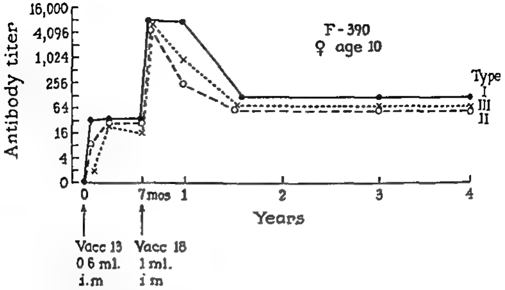

Fig 81 Degree of persistence of antibody over a 4-year period (Salk, J E, 1958, How many injections of poliomyelitis vaccine are required for effective and durable immunity? J A M A *167*, 1-7.

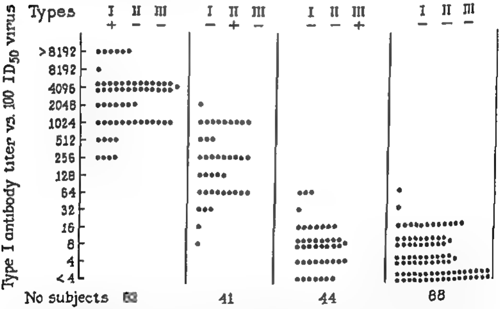

Fig 82. Type I antibody levels after 1 dose of vaccine in children with different prevaccination antibody patterns to illustrate significantly greater type I response in individuals who have had a prior type II infection in comparison with a lesser response in individuals who have had a prior type III infection.

+ = Antibody demonstrable before vaccination at titer of 1:4 or > as determined by color test for antibody measurement in tissue culture (Salk, Youngner, Ward, 1954)

(Salk, J. E., 1956, Requirements for persistent immunity to poliomyelitis, Am.J M.Sc. *232*, 369-377)

dicate that the relatively more rapid decline after the postinjection peak is followed by a very gradual decline or a plateau This suggests that antibody could be expected to be present for several years at least in persons in whom a sufficient degree of antigenic stimulation was once induced The full length of such persistence will be established in time

Effect of Prior Immunologic Experience

It is to be expected that persons who have had a prior natural infection would respond to vaccination with much higher levels of homotypic antibody than would others who had had no prior experience with the antigen in question Figure 82 illustrates the greater response to the type I component of the vaccine in persons with pre-existing type I antibody as compared with others with no prior antibody to any of the 3 types Figure 82 also illustrates the rather unexpected finding that individuals who, prior to vaccination, have only type II antibody from a natural infection, respond with the formation of higher levels of antibody to the type I component of the vaccine than do individuals who have had a prior type III infection (Salk, 1955a, 1956a) These data clearly suggest that the type II viruses that were the cause of these infections had sensitized the immunologic mechanism to type I antigen The response in persons in whom type III infections had occurred previous to vaccination revealed such effects to only a very slight degree. From these and other data it appears that a significant degree of cross relationship exists between types II and I, and types II and III, and to a lesser degree or none at all, between I and III. It is clear from such observations, which have been confirmed (Francis et al, 1957), that a prior natural infection facilitates the formation of antibody not only for the prior infecting type but for other types in proportion to the degree of cross relationship

Observation of Antibody Response in Large Groups and Exceptional Effects

The extent to which antibody formation has been induced in a large group of human subjects is revealed by a study involving a population of 4,617 children, 847 of whom had no demonstrable antibody to any of the 3 types, at a screening level of 1 64 (Salk, 1956c) This was determined by a test performed on capillary blood obtained from a finger-prick After 3 doses of vaccine with an interval of 2 weeks between first and second doses and approximately 1 year between the second and third doses, 803 of the 847 had antibody levels of 1 64 or greater for

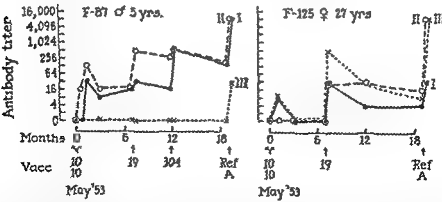

Fig 83 Individual variation in response to the 3 antigens The relatively poor response to type III, illustrated in the case of this particular child, is contrasted with the relative poor response to type I in this particular adult (Salk, J E, 1958, Basic principles underlying immunization against poliomyelitis with a noninfectious vaccine in Poliomyelitis· Papers and Discussions Presented at the Fourth International Poliomyelitis Conference, Philadelphia, Lippincott)

all 3 types, of the remaining 44, all reacted with antibody formation to a level of 1:64 or greater for type II, and lower levels of antibody for types I and III were present in all but one instance It is clear from these observations that all of the children involved were capable of forming antibody if stimulated by a sufficient concentration of antigen The lesser response to types I and III reflect, in these instances, lesser antigenic potencies of the types I and III components of certain of the lots of vaccine involved in this study

In the course of extensive serologic studies involving about 15,000 persons, mostly children but including some adults, rare instances have been observed in which an individual appears to be poorly responsive, or relatively refractory, to 1 of the 3 antigenic components of the vaccines employed The reasons for these unusual effects are not clear, but the facts are illustrated in Figure 83 Here it may be seen that a child who responded well to types I and II, but not to type III, reacted differently from another individual who reacted poorly to type I but appeared to react in the expected fashion to the type III component to which the child was nonreactive The same lots of vaccine were not used throughout the course for both individuals, but there was sufficient similarity for this comparison These extreme examples may reflect either an inborn or an acquired refractoriness to certain antigens, possibly based on the same mechanism as similar examples in other antigen systems

IMMUNOLOGIC FACTORS INVOLVED IN THE IMMUNITY MECHANISM

From the nature of the pathogenesis of the paralytic disease, it is readily understandable that antibody in the serum would be effective in protecting the central nervous system from invasion by virus and in this way prevent paralysis. However, there are some individuals in whom paralysis does not occur even though specific serum antibody was not present before exposure to infection In such instances it might be said that the virus may have been essentially noninvasive. However, certain observations provide a basis for doubt that this is the full explanation The first observation to arrest attention is the greater frequency with which type I paralytic polio occurs in persons who have had an earlier type III infection, as compared with individuals who have had a prior type II infection (Salk, 1955a; 1956a; Hammon, and Ludwig, 1957). The mechanism for protection against paralysis could be the result of the presence of type I antigen in type II viruses (Melnick, 1955) and due to the effect of nondemonstrable type I antibody in the serum, as sometimes does appear in convalescence from type II infections in man (Sabin, 1952). However, the fact that type I antibody activity may not be readily demonstrable makes it necessary to examine whether or not such activity is actually present and, therefore, whether the foregoing explanation is sufficient. Perhaps other factors may be operative.

Further pursuit of this question is possible because of the observations of the difference in type I antibody response in vaccinated persons who have had a prior type II infection as compared with those who have had a prior type III infection (Salk, 1955a) Figure 82 illustrates this difference and suggests the existence of significant type I immunologic hyper-reactivity in those who have had a prior type II infection, in comparison with those who have had a prior type III infection The question then arises as to whether or not a relationship may exist between the protective effect against type I paralysis among those who have had a prior type II infection and their heightened reactivity to type I antigen

The phenomenon of immunologic hyper-reactivity, or the booster phenomenon in immunology, is associated not only with a greatly increased production of antibody but also with earlier appearance of greater concentrations of antibody in the serum Regardless of whether the hyper-reactive mechanism results in the more rapid appearance of antibody or merely a greater production, there is the reasonable question as to whether or not heightened immunologic reactivity could play a role in prevention of paralysis, under circumstances where the incubation period before paralysis is such as to permit antibody to be effective in reducing the probability of paralysis. From studies on the pathogenesis

of paralytic polio, Bodian (cf Chap. 23) has pointed out that there is an incubation period of approximately 7 days from exposure to onset of systemic symptoms, and approximately 5 days between them and onset of neural symptoms This suggests an interval of between 7 and 12 days from exposure to the initiation of CNS infection MacLeod (1953) has proposed a theory to explain lifelong immunity in certain infectious diseases and not in others He has reasoned that the earlier appearance of effective concentrations of antibody in an individual who has had a prior immunologic experience, coupled with a sufficiently long incubation period to allow this effect to be operative, could explain lifelong immunity to diseases of long incubation and relatively short-term immunity in diseases of short incubation period Whether or not this theory is generally applicable or can be considered to apply in this instance will be established in the course of time

In the light of the foregoing it is interesting to view the observation (Lau et al, 1957) that injections of relatively large amounts of antiserum into monkeys in which a virulent strain of virus is also inoculated into the lumbar segment of the cord were associated with the prevention of further spread within the CNS even though antibody was not administered until 24 to 48 hours after the intraspinal injection of virus This suggests the possibility that the development of high concentrations of antibody, even after CNS invasion by virus, could result in limitation of virus spread and could conceivably, in some instances, convert a potentially destructive CNS invasion into one that could be self-reversing and without crippling effect Thus, it is possible to imagine that the degree of immunologic hyper-reactivity that might persist after vaccination, if operative after onset of infection, could either prevent or ameliorate the effects of CNS invasion, depending upon the time lapse between virus invasion and rise in concentration of antibody

The delicate time-balance between the effect of the invasive properties of the virus and the immunologic defense of the host could be the critical factor that determines whether or not paralysis will occur, and it is conceivable that this mechanism could operate even without a prior antigenic stimulus However, the hypothesis proposed is that prior immunologic stimulation places the host at an advantage, more so if antibody is present in the serum, or at an advantage that is in proportion to the degree to which antibody can be produced before extensive and irreversible effects have occurred in the CNS This concept, although plausible, is still hypothetical and provides a convenient way of regarding the balance between pathogenic and immunogenic forces as these could influence the encounter between virus and host It also provides a way of regarding the role of immunization and of the way in which the important quantitative and qualitative factors must be manipulated to produce a desired effect

Resistance to Infection vs Resistance to Paralysis

There are many circumstances under which infection can occur which does not result in paralysis (1) Passively transferred antibody in the infant suppresses paralysis but does not inhibit infection (2) The resistance to paralysis induced by a cross-related heterotypic infection is not accompanied by resistance to infection (Salk, 1955a, 1956a) (3) It has been shown that primary vaccination is able to induce resistance to paralysis, but this does not appear to prevent the establishment of infection resulting either from feeding of attenuated viruses (Sabin, 1957) or that naturally acquired (Gelfand et al, 1956)

However, the consequences of natural infection, or of infection induced artificially by feeding attenuated virus, does result in resistance to reinfection in a high proportion of persons The fact that such resistance does not necessarily exist in all who had previously been infected suggests that there is a qualitative component to the immune mechanism that could conceivably be simulated by an immunizing procedure that does not necessitate the process of infection For example, Howe (1955) has shown that high titers of antibody, resulting from repeated inoculations of chimpanzees with formalin-treated virus, is accompanied by limitation in viral excretion after feeding live-virus, or to re-

all 3 types; of the remaining 44, all reacted with antibody formation to a level of 1:64 or greater for type II, and lower levels of antibody for types I and III were present in all but one instance It is clear from these observations that all of the children involved were capable of forming antibody if stimulated by a sufficient concentration of antigen. The lesser response to types I and III reflect, in these instances, lesser antigenic potencies of the types I and III components of certain of the lots of vaccine involved in this study

In the course of extensive serologic studies involving about 15,000 persons, mostly children but including some adults, rare instances have been observed in which an individual appears to be poorly responsive, or relatively refractory, to 1 of the 3 antigenic components of the vaccines employed The reasons for these unusual effects are not clear, but the facts are illustrated in Figure 83 Here it may be seen that a child who responded well to types I and II, but not to type III, reacted differently from another individual who reacted poorly to type I but appeared to react in the expected fashion to the type III component to which the child was nonreactive The same lots of vaccine were not used throughout the course for both individuals, but there was sufficient similarity for this comparison These extreme examples may reflect either an inborn or an acquired refractoriness to certain antigens, possibly based on the same mechanism as similar examples in other antigen systems

IMMUNOLOGIC FACTORS INVOLVED IN THE IMMUNITY MECHANISM

From the nature of the pathogenesis of the paralytic disease, it is readily understandable that antibody in the serum would be effective in protecting the central nervous system from invasion by virus and in this way prevent paralysis However, there are some individuals in whom paralysis does not occur even though specific serum antibody was not present before exposure to infection In such instances it might be said that the virus may have been essentially noninvasive However, certain observations provide a basis for doubt that this is the full explanation. The first observation to arrest attention is the greater frequency with which type I paralytic polio occurs in persons who have had an earlier type III infection, as compared with individuals who have had a prior type II infection (Salk, 1955a; 1956a; Hammon, and Ludwig, 1957). The mechanism for protection against paralysis could be the result of the presence of type I antigen in type II viruses (Melnick, 1955) and due to the effect of nondemonstrable type I antibody in the serum, as sometimes does appear in convalescence from type II infections in man (Sabin, 1952) However, the fact that type I antibody activity may not be readily demonstrable makes it necessary to examine whether or not such activity is actually present and, therefore, whether the foregoing explanation is sufficient. Perhaps other factors may be operative.

Further pursuit of this question is possible because of the observations of the difference in type I antibody response in vaccinated persons who have had a prior type II infection as compared with those who have had a prior type III infection (Salk, 1955a). Figure ■ illustrates this difference and suggests the existence of significant type I immunologic hyper-reactivity in those who have had a prior type II infection, in comparison with those who have had a prior type III infection The question then arises as to whether or not a relationship may exist between the protective effect against type I paralysis among those who have had a prior type II infection and their heightened reactivity to type I antigen

The phenomenon of immunologic hyper-reactivity, or the booster phenomenon in immunology, is associated not only with a greatly increased production of antibody but also with earlier appearance of greater concentrations of antibody in the serum Regardless of whether the hyper-reactive mechanism results in the more rapid appearance of antibody or merely a greater production, there is the reasonable question as to whether or not heightened immunologic reactivity could play a role in prevention of paralysis, under circumstances where the incubation period before paralysis is such as to permit antibody to be effective in reducing the probability of paralysis. From studies on the pathogenesis

formation consistently exerted a more complete effect, and antigenically poor lots were demonstrated to have been relatively ineffective in preventing paralysis by the type for which there was antigenic deficiency Francis et al (1957) concluded that, "These studies leave little doubt that the effectiveness of the vaccines in the Field Trial of 1954 was closely associated with, perhaps entirely dependent upon, the ability of the lots of vaccine used to produce discernible type-specific antibody rise in children who previously had no discernible type-specific antibody" From data on type I vaccine effectiveness, it appeared that practically all type I paralytic cases in vaccinated children occurred in groups given lots of vaccine in which antigenic effectiveness was computed to be less than 70 per cent A vaccine that was 70 per cent effective antigenically was one that induced the formation of antibody levels (of 1 4 or greater, when tested against 100 ID_{50} of virus) in 70 per cent of children who previously had no discernible type-specific antibody. Thus, it was observed that vaccines that exceeded 70 per cent effectiveness in inducing antibody formation approached 100 per cent effectiveness in resistance to paralysis

In the Francis et al report (1957), detailed data are presented on questions of reactions and of safety, as well as the question of provocation of paralysis by inoculation There was no evidence of unsafe vaccine, in the sense of infection induced by the vaccine, nor evidence of provocation The administration of vaccine seemed to have been essentially free of associated side-reactions

Experience Subsequent to Field Trial

Among the first lots of vaccine released for general use in the United States, in April, 1955, there were several, prepared by one manufacturer, that were associated with the development of paralytic poliomyelitis in inoculated children (Langmuir et al, 1956) In

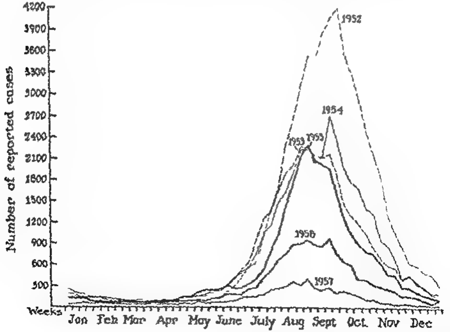

FIG 84 Weekly United States Poliomyelitis Incidence 1952-1957 Data provided by National Office Vital Statistics and chart reproduced with permission of Poliomyelitis Surveillance Unit, Communicable Disease Center, United States Public Health Service Data are total reported cases including paralytic and nonparalytic.

sistance to the establishment of the carrier state.

Whether or not, and to what degree, vaccination of man with a killed-virus vaccine will affect the rate of poliovirus infection, as distinct from the paralytic rate, after high concentrations of antibody are induced, will require further study. If experience in the course of widespread application indicates a reduction in poliomyelitis out of proportion to the number of individuals injected and if there is a reduction in nonparalytic, as well as in paralytic cases, and if there is no evidence that vaccination affects the fecal carrier state, then it becomes necessary to consider the possibility that the fecal source of virus may be less important in the spread of this disease and, as suggested by Rivers, that some other source, such as the pharynx, may be more important. Vaccination might possibly have an effect upon the pharyngeal source of virus dissemination while influencing to a lesser degree the fecal source. In this regard, it is known that virus disappears from the pharynx within approximately 1 week after onset of symptoms, but that virus can still be found in feces for weeks and sometimes months later. Fecal virus persists in spite of the presence of good concentrations of serum antibody; the latter appears to remain at a fixed level or to decline slightly in spite of the presence of virus in the intestine. The nature of this state is not clearly understood.

It is generally believed that exposure of contacts during the latter part of the incubation period, or within 7 days of onset of symptoms, is the kind that results in spread of infection, with less likelihood of ready spread resulting from later contacts, when the only source of virus from the infected or convalescent carrier is bowel excreta. The latter source of infection may be of greater importance under circumstances of poor community hygiene, or contamination of food and water, or by improper sewage disposal.

Field Trial of 1954

In a remarkable study, probably never again to be repeated for any disease, Francis (Francis et al., 1957) directed a field evaluation of vaccination against poliomyelitis. The details of this study, to the report of which the reader is referred, are too numerous to present and will be indicated here only in essence.

Two types of observations were made: one in which volunteer children in the first 3 grades of school were randomly divided into alternate individuals to whom either vaccine or a placebo injection was administered. In other areas, vaccine was given to volunteer children in the second grade and comparisons were made with nonvolunteer children, in the same grade, who were not vaccinated and with nonvaccinated but recorded volunteer children in the first and the third grades. A total of 1,829,916 individuals were under observation during the period of study. In the strictly controlled placebo study 200,745 children received 3 doses of vaccine, and 201,229 received 3 doses of the placebo injection. In the observed study 221,998 children in the second grade received 3 doses of vaccine. The 3 doses of vaccine were given within an interval of 5 weeks, the first 2 doses 1 week apart and the second 4 weeks later.

Under the circumstances of primary vaccination, without the added advantage of the secondary stimulus, for which a longer interval is required, and the added circumstance that the preservative used in the vaccine had caused a definite but variable deterioration of the type I component of the vaccine, and deterioration to a lesser extent of the type II and type III components, there was demonstrated an over-all reduction of 70 per cent in paralytic disease in the Placebo Control Study and 62 per cent in the Observed Control Study. Analysis only of cases in which laboratory confirmation of diagnosis could be established by virus isolation recorded the degree of effectiveness in the respective studies as 82 per cent and 76 per cent. The virus positive cases in the Placebo Study revealed the following degrees of effectiveness in preventing paralysis for the respective types. I, 71 per cent, II, 100 per cent, and III, 95 per cent. The same distinctions in the Observed Study revealed 71 per cent for type I; 75 per cent for types II and III.

It was clear that the effectiveness of the vaccine employed was in proportion to the demonstrated antigenic potency of the particular lot—vaccines that induced antibody

Bodian, D, Morgan, I M, and Howe, H A, 1949, Differentiation of types of poliomyelitis viruses III The grouping of fourteen strains into three basic immunological types Am J Hyg 49, 234-245

Boyd, T E, 1953, *Immunization against poliomyelitis*, Bact Rev 17, 339-448

——, 1957, Part I Poliomyelitis a biography of poliomyelitis in Cellular Biology, Nucleic Acids and Viruses (Special publication) vol 5, pp 39-56, New York Academy of Sciences

Brodie, M, and Park, W H, 1936, Active immunization against poliomyelitis, Am J Pub Health 26, 119-125

Burnet, F M, and Macnamara, J, 1931, Immunological differences between strains of poliomyelitis virus, Brit J Exper Path 12, 57-61

Dane, D S, Dick, G W, Fisher, O D, Connolly, J H, and McKeown, F, 1957, Vaccination against poliomyelitis with live virus vaccines I A trial of TN type II vaccine Brit M J 1, 59 65

Dick, G W, and Dane, D S, 1957, Vaccination against poliomyelitis with live virus vaccines III The evaluation of TN and SM virus vaccines Brit M J 1, 70-74

Dick, G W, Dane, D S, Fisher, O D, Connolly, J H, and McKeown, F, 1957, Vaccination against poliomyelitis with live virus vaccines II A trial of SM type I attenuated poliomyelitis virus vaccine Brit M J 1, 65-70

Enders, J F, Weller, T H, and Robbins, F C, 1949, Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues, Science 109, 85-87

Fox, J P, 1957, Part I Poliomyelitis Discussion of immunization against poliomyelitis killed vaccine followed by induced infection with live virus in Cellular Biology, Nucleic Acids and Viruses (Special publication) vol 5, pp 152-155, New York Academy of Sciences

Francis, T, Jr, et al, 1957, Evaluation of the 1954 field trial of poliomyelitis vaccine (final report), Michigan University, Poliomyelitis Vaccine Evaluation Center

Gard, S, and Lycke, E, 1957, Inactivation of poliovirus by formaldehyde Analysis of inactivation curves, Arch ges Virusforsch 7, 471-482

Gelfand, H M, Fox, J P, and LeBlanc, D R, 19[illegible]

[illegible]

and Stokes, J, Jr, 1953, Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis 4 Final report of results based on clinical diagnoses J A M A 151, 1271-1285.

Hammon, W McD, and Ludwig, E H, 1957, Possible protective effect of previous type 2 infection against paralytic poliomyelitis due to type 1 virus, Am J Hyg 66, 274

Howe, H A, 1952a, Duration of immunity in chimpanzees vaccinated with formalin inactivated poliomyelitis virus, Fed Proc 11, 471-472

—— 1952b, Antibody response of chimpanzees and human beings to formalin inactivated trivalent poliomyelitis vaccine, Am J Hyg 56, 265-286.

——, 1955, Poliomyelitis infection in immunized chimpanzees, Ann New York Acad Sc 61, 1014-1022

Kessel, J F, and Pait, C F, 1949, Differentiation of three groups of poliomyelitis virus, Proc Soc Exper Biol & Med 70, 315-316

Kolmer, J A, 1936, Vaccination against acute anterior poliomyelitis, Am J Pub Health 26, 126-135

Koprowski, H, 1957, Discussion of properties of attenuated polioviruses and their behavior in human beings in Cellular Biology, Nucleic Acids and [illegible]

[illegible]

Department of Health, Education and Welfare, Public Health Service, Bureau of State Services

Langmuir, A D, Nathanson, N, and Hall, W J, 1956, Surveillance of poliomyelitis in the United States in 1955, Am J Pub Health 46, 75-88

Liu, O C, Carter, J E, and Hampil, B, 1957, Serotherapy of poliomyelitis in rhesus monkeys, Fed Proc 16, 423

Loring, H S, Schwerdt, C E, Lawrence, N, and Anderson, J C, 1947, Preparation of formaldehyde-inactivated poliomyelitis virus and its use as an immunizing agent in cotton rats, Science 106, 104 105

MacLeod, C M, 1953, Relation of the incubation period and the secondary immune response to lasting immunity in infectious diseases, J Immunol 70, 421-425

Melnick, J L, 1955, Antigenic crossings within poliovirus types, Proc Soc Exper Biol & Med 89, 131-133

Milzer, A, Oppenheimer, F, and Levinson, S O, 1945, A new method for the production of potent inactivated vaccines with ultraviolet irradiation III A completely inactivated poliomyelitis vaccine with the Lansing strain in mice J Immunol 50, 331-339

Morgan, I M, 1948, Immunization of monkeys with formalin inactivated poliomyelitis viruses, Am J Hyg 48, 394-406

National Foundation for Infantile Paralysis, Committee on Typing, 1951, Immunologic classification of poliomyelitis viruses I A cooperative program for the typing of one hundred strains Am J Hyg 54, 191-204

Paul, J R, Horstmann, D M, Melnick, J L, Niederman, J C, and Deutsch, J, 1957, Immunization against poliomyelitis killed vaccine followed by induced infection with live virus in Cellular Biology, Nucleic Acids and Viruses (Special pub-

addition, family associates, and other contacts of children given vaccine from the implicated lots, developed paralytic poliomyelitis Further use of vaccine prepared by this manufacturer was discontinued, and after review and reconsideration, the general use of vaccine prepared by other manufacturers was continued Although supply was limited, use of vaccine was sufficiently extensive to reveal a distinctly protective effect, even among individuals given a single dose of vaccine (Langmuir et al., 1956). Further use of vaccine in 1956 and 1957 seems to have been associated with an over-all reduction in incidence of disease in the United States (Langmuir, 1957) beyond that accountable for merely on the basis of the number of individuals inoculated, in whom the incidence of disease is lower than in the nonvaccinated Some have regarded this as an indication of vaccine effect upon the reservoir of infection, although it is cautioned that this may merely represent normal variation in a fluctuating incidence The experience in the United States over the years 1952-1957 is shown in Figure 84.

In a number of smaller countries in which the incidence of paralytic poliomyelitis is generally restricted to the youngest age group, and where populations are relatively small, the use of vaccine appears to have been associated with a very sharp reduction in occurrence of this disease

SUMMARY OF PRACTICAL IMPLICATIONS

On the basis of current knowledge and experience, the following suggestions may be made for use of vaccine for the control of paralytic poliomyelitis·

The vaccine employed should be of such potency that when 2 doses are administered, by whatever route, within an interval of approximately 1 month, the formation of neutralizing antibody (neutralization of 100 ID_{50} by approximately 0 1 ml of serum) is elicited in at least 75 per cent of children who have had no prior contact with poliovirus infection

A third dose of vaccine should be administered 6 to 7 months or longer thereafter. Satisfactory responses have been elicited at shorter intervals of 3 to 4 months when tested because the interval available before the ensuing poliomyelitis season was short; the longer interval provides greater opportunity for development of the hyper-reactive state.

In countries where paralytic poliomyelitis does occur in adults, it would be desirable to immunize mothers, preferably before conception, or early in pregnancy In this way, the relatively more exposed young mother who is also more susceptible in pregnancy, is protected; and the fetus is also protected if the mother might otherwise experience an infection with viremia.

The infant should be immunized before maternal antibody is lost, i e, between 2 and 6 months of age (Thrupp, 1958). It is probable that the means for immunization against poliomyelitis and against diphtheria, tetanus and pertussis may soon be combined or, at least, be practiced simultaneously.

Further observations will be required to determine the duration of protection and the need for further inoculations It is clear that antibody is not evanescent and that immunologic hyper-reactivity persists for a considerable period and possibly for life after effective immunization has once been induced

In countries in which the age distribution of paralytic poliomyelitis is low, the disease can be controlled by immunization of the affected age groups first and then, as supplies of vaccine are available, to extend the use of vaccine to those in older age groups, to protect those few who may have escaped natural immunization in their earlier years

REFERENCES

Bodian, D, 1951, Experimental studies on passive immunization against poliomyelitis I Protection with human gamma globulin against intramuscular inoculation, and combined passive and active immunization Am J Hyg *54*, 132-143

Bodian, D, for Technical Committee on Poliomyelitis Vaccine, 1958, Control of the manufacture of poliomyelitis vaccine *in* Poliomyelitis Papers and Discussions Presented at the Fourth International Poliomyelitis Conference, pp 77-85, Philadelphia, Lippincott

Bodian, D, Francis, T. Jr, Larson, C, Salk, J E, Shope, R E, Smadel, J E, and Shannon, J A, 1955, Interim report of Public Health Service Technical Committee on Poliomyelitis Vaccine J.A.M.A *159*, 1444-1447.

Bodian, D, Morgan, I M, and Howe, H A, 1949, Differentiation of types of poliomyelitis viruses III The grouping of fourteen strains into three basic immunological types, Am J Hyg *49*, 234-245

Boyd, T E, 1953, Immunization against poliomyelitis, Bact Rev *17*, 339-448

———, 1957, Part I Poliomyelitis a biography of poliomyelitis *in* Cellular Biology, Nucleic Acids and Viruses (Special publication) vol 5, pp 39-56, New York Academy of Sciences

Brodie, M, and Park, W H, 1936, Active immunization against poliomyelitis, Am J Pub Health *26*, 119-125

Burnet, F M, and Macnamara, J, 1931, Immunological differences between strains of poliomyelitis virus, Brit J Exper Path *12*, 57-61

Dane, D S, Dick, G W, Fisher, O D, Connolly, J H, and McKeown, F, 1957, Vaccination against poliomyelitis with live virus vaccines 1 A trial of TN type II vaccine Brit M J *1*, 59-65

Dick, G W, and Dane, D S, 1957, Vaccination against poliomyelitis with live virus vaccines III The evaluation of TN and SM virus vaccines Brit M J *1*, 70-74

Dick, G W, Dane, D S, Fisher, O D, Connolly, J H, and McKeown, F, 1957, Vaccination against poliomyelitis with live virus vaccines II A trial of SM type I attenuated poliomyelitis virus vaccine Brit M J *1*, 65-70

Enders, J F, Weller, T H, and Robbins, F C, 1949, Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues, Science *109*, 85-87

Fox, J P, 1957, Part I Poliomyelitis Discussion of [illegible]

Academy of Sciences

Francis, T, Jr, et al, 1957, Evaluation of the 1954 field trial of poliomyelitis vaccine (final report), Michigan University, Poliomyelitis Vaccine Evaluation Center

Gard, S, and Lycke, E, 1957, Inactivation of poliovirus by formaldehyde Analysis of inactivation curves, Arch ges Virusforsch *7*, 471-482

Gelfand, H M, Fox, J P, and LeBlanc, D R, 1956, Observations on natural poliomyelitis infection in immunized children, Pub Health Assn Mtg, Unpublished

Hammon, W McD, Corell, L L, Wehrle, P F, and Stokes, J, Jr, 1953, Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis 4 Final report of results based on clinical diagnoses J.A.M.A *151*, 1272-1285

Hammon, W McD, and Ludwig, E H, 1957, Possible protective effect of previous type 2 infection against paralytic poliomyelitis due to type 1 virus, Am J Hyg *66*, 274

Howe, H A, 1952a, Duration of immunity in chimpanzees vaccinated with formalin inactivated poliomyelitis virus, Fed Proc *11*, 471-472.

———, 1952b, Antibody response of chimpanzees and human beings to formalin-inactivated trivalent poliomyelitis vaccine, Am J Hyg *56*, 265-286

———, 1955, Poliomyelitis infection in immunized chimpanzees, Ann New York Acad Sc *61*, 1014-1022

Kessel, J F, and Pait, C F, 1949, Differentiation of three groups of poliomyelitis virus, Proc Soc Exper Biol & Med *70*, 315-316

Kolmer, J A, 1936, Vaccination against acute anterior poliomyelitis, Am J Pub Health *26*, 126-135

Koprowski, H, 1957, Discussion of properties of attenuated polioviruses and their behavior in human beings *in* Cellular Biology, Nucleic Acids and Viruses (Special publication) vol 5, pp 128-133, New York Academy of Sciences

Koprowski, H, Jervis, G A, and Norton, T. W, 1952, Immune responses in human volunteers upon [illegible]

L[illegible]

L[illegible]

Public Health Service, Bureau of State Services

Langmuir, A D, Nathanson, N, and Hall, W J, 1956, Surveillance of poliomyelitis in the United States in 1955, Am J Pub Health *46*, 75-88

Liu, O C, Carter, J E, and Hampil, B, 1957, Serotherapy of poliomyelitis in rhesus monkeys, Fed Proc *16*, 423

Loring, H S, Schwerdt, C E, Lawrence, N, and Anderson, J C, 1947, Preparation of formaldehyde-inactivated poliomyelitis virus and its use as an immunizing agent in cotton rats, Science *106*, 104-105

MacLeod, C M, 1953, Relation of the incubation period and the secondary immune response to lasting immunity to infectious diseases, J Immunol *70*, 421-425

Melnick, J L, 1955, Antigenic crossings within poliovirus types, Proc Soc Exper Biol & Med *89*, 131-133

Milzer, A, Oppenheimer, F, and Levinson, S O, 1945, A new method for the production of potent inactivated vaccines with ultraviolet irradiation III A completely inactivated poliomyelitis vaccine with the Lansing strain in mice J Immunol. *50*, 331-339

Morgan, I M, 1948, Immunization of monkeys with formalin inactivated poliomyelitis viruses, Am J Hyg *48*, 394-406

National Foundation for Infantile Paralysis, Committee on Typing, 1951, Immunologic classification of poliomyelitis viruses I A cooperative program for the typing of one hundred strains Am J Hyg *54*, 191-204

Paul, J R, Horstmann, D M, Melnick, J L, Niederman, J C, and Deutsch, J, 1957, Immunization against poliomyelitis killed vaccine followed by induced infection with live virus *in* Cellular Biology, Nucleic Acids and Viruses (Special pub-

lication) vol 5, pp 141-147, New York Academy of Sciences

Paul, J R, and Riordan, J. R, 1950, Observations on serologic epidemiology Antibodies to the Lansing strain of poliomyelitis virus in sera from Alaskan eskimos Am J Hyg *52, 202-212*

Rivers, T M, Personal communication

Sabin, A. B, 1952, Transitory appearance of type 2 neutralizing antibody in patients infected with type 1 poliomyelitis virus J Exper Med. *96,* 99-106.

———, 1957, Properties of attenuated polioviruses and their behavior in human beings *in* Cellular Biology, Nucleic Acids and Viruses (Special publication) vol *5,* pp 113-127, New York Academy of Sciences

Salk, J E, 1955a, A concept of the mechanism of immunity for preventing paralysis in poliomyelitis, Ann New York Acad Sc *61, 1023-1038*

———, 1955b, Considerations in the preparation and use of poliomyelitis virus vaccine, J A M A *158, 1239-1248*

———, 1956a, Requirements for persistent immunity to poliomyelitis, Am J M Sc *232, 369-377.*

———, 1956b, Poliomyelitis vaccine in the fall of 1955, Am J Pub Health *46,* 1-14

———, 1956c, Antigenic potency of poliomyelitis vaccine Influence on degree and duration of vaccine effect J A M A *162, 1451-1459*

———, 1957a, Viral and cellular factors pertinent to the control of paralytic poliomyelitis with a noninfectious vaccine *in* Cellular Biology, Nucleic Acids, and Viruses (Special publication) vol *5,* pp 77-89, New York Academy of Sciences

———, 1957b, Poliomyelitis vaccination in the fall of 1956, Am J Pub Health *47, 1-18*

———, 1958, Basic principles underlying immunization against poliomyelitis with a noninfectious vaccine *in* Poliomyelitis Papers and Discussions Presented at Fourth International Poliomyelitis Conference, pp 66-76, Philadelphia, Lippincott

Salk, J. E, with the collaboration of Bennett, B L., Lewis, L J., Ward, E N, and Youngner, J S, 1953, Studies in human subjects on active immunization against poliomyelitis I. A preliminary report of experiments in progress J A M A *151, 1081-1098*

Salk, J E, Krech, U., Youngner, J S, Bennett, B L, Lewis, L J, and Bazeley, P L, 1954, Formaldehyde treatment and safety testing of experimental poliomyelitis vaccines, Am J Pub Health *44, 563-570*

Salk, J E, Lewis, L J, Bennett, B L, Ward, E N, Krech, U., Youngner, J S, and Bazeley, P L, 1955, Antigenic activity of poliomyelitis vaccines undergoing field test, Am J Pub Health *45, 151-162*

Salk, J E, Youngner, J S, and Ward, E N., 1954, Use of color change of phenol red as the indicator in titrating poliomyelitis virus or its antibody in a tissue-culture system Am J Hyg *60, 214-230*

Schwerdt, C E, Dick, G W A, Herriott, R M, and Howe, H A, 1951, Immunization of cotton rats with inactivated Lansing poliomyelitis virus I. Inactivation by chemical agents Am J Hyg *53,* 121-130

Smith, W M, Chambers, V C., and Evans, C A, 1950, Growth of neurotropic viruses in extraneural tissues, preliminary report on propagation of poliomyelitis virus (Lansing and Hof strains) in cultures of human testicular tissue Northwest Med *49, 368-373.*

Taylor, A R., Kay, W W., McLean, I W, Jr, Oppenheimer, F, and Stimpert, F D, 1957, Effect of ultraviolet light on poliomyelitis virus, J Immunol *78, 45-55.*

Timm, E A, McLean, I W, Jr, Kupsky, C H, and Hook, A E, 1956, The nature of the formalin inactivation of poliomyelitis virus, J Immunol *77,* 444-452

Thrupp, L D, 1958, Immunization of infants with poliomyelitis vaccine, J A M A *166,* 160-161

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25

The Coxsackie Virus Group

INTRODUCTION

The Coxsackie viruses comprise 2 groups of small agents having similar physical and biologic properties. Their most striking biologic property is pathogenicity for newborn mice and hamsters and the nature of the lesions which permit their identification and assignment to Group A or B. Throughout the world they are common enteric infections in man. Their seasonal and geographic distribution is not unlike that of the polioviruses. They cause a variety of illnesses, including several clinically distinct ones.

HISTORY

The first strains of what are now known as the Coxsackie viruses were isolated from the feces of 2 boys suffering from paralytic poliomyelitis (Dalldorf and Sickles, 1948). They were the first evidence of the existence of the large and growing number of hitherto unsuspected enteric agents currently subdivided into Coxsackie and ECHO viruses. The original interest in the Coxsackie viruses was related to their association with and simi- [illegible]

became known as the Group-B viruses (Dalldorf, 1950) were isolated, and it was suggested that the latter might induce aseptic meningitis and be responsible for epidemic myalgia or Bornholm disease (Curnen et al., 1949). This was confirmed the following year (Weller et al., 1950; Kilbourne, 1950). In 1951, evidence was secured connecting certain Group-A viruses with herpangina (Huebner et al., 1951) and 2 years later a Group-B [illegible]

demonstration that strains representing 2 Group-A types are capable of causing poliomyelitislike lesions in monkeys (Dalldorf, 1957a) and the claim that one of these was [illegible]

since too little was known of the pathogenesis of human infections to justify a descriptive term (Dalldorf, 1949a). Coxsackie is the name of the village in which the outbreak occurred from which the original isolations were made.

CLINICAL PICTURE

The incubation period of infection by viruses of the Coxsackie group appears to range

from 1 to 14 days with a mean between 3 and 5 (Curnen, 1950, Findlay and Howard, 1950; Huebner et al, 1951, Warin et al., 1953)

The manifestations of infection in man by individual Coxsackie viruses are not uniform, but the association of some of these viruses with particular disease entities has been recognized to represent a causal relationship Classification of Coxsackie viruses into Groups A and B, based on the character and the distribution of lesions experimentally induced in suckling mice, has useful application in considering the relations of these agents to human disease. Five antigenically distinct viral "types" of Group B and 23 of Group A have been identified All of the Group-B viruses and approximately half in the Group-A category have been shown to cause clinically recognizable disease. Information concerning the clinical significance of other Group-A types is still equivocal or lacking Meanwhile, there is no assurance that the growing list of Coxsackie viruses is yet complete. Moreover, recent recognition that certain viruses have properties common to both the Coxsackie and other viral groups has made classification of these agents and delineation of their relation to associated disease more, rather than less, complex (Dalldorf, 1957b, Melnick, 1957b) At present the clinical picture of infection by Coxsackie viruses might be likened to a painting in which important features are clearly delineated but many details remain indistinct. Therefore, an attempt at this time to present a comprehensive description of infection by Coxsackie viruses would still be premature Nevertheless, it is possible to point out certain diseases which are caused by particular Coxsackie viruses and to indicate others in which the etiologic relation of virus to disease is suggested but less firmly established.

Aseptic Meningitis

It has become increasingly evident that Coxsackie viruses of Group B, Types 1 to 5 not only are capable of causing meningitis but periodically and seasonally in summer and fall are probably responsible for a considerable proportion of cases assigned a clinical diagnosis of aseptic meningitis or "nonparalytic" poliomyelitis Initially, the evidence for an etiologic relationship was indirect, based on epidemiologic and clinical findings, recovery of virus from feces and pharyngeal swabbings of patients, detection of serologic response to infection with the homologous strain, and failure to demonstrate evidence of infection with poliomyelitis, mumps, or other virus (Curnen et al, 1949; Melnick et al., 1950; Gard and Johnsson, 1952, Rhodes et al, 1953; Stanley et al., 1953; Gard, 1954; Johnsson, 1954, 1955a, b, c, d; Wilkins et al, 1955, Bayer and Gear, 1955, Syverton et al., 1957).

Possibly of some additional value as evidence is the accumulating experience that during interepidemic periods these agents are infrequently found in the feces of children who are healthy or afflicted with other common diseases (Cole et al., 1951; Ramos-Alvarez and Sabin, 1954, Parrott et al, 1955, Johnsson, 1955c; Rhodes and Beale, 1957) and have been encountered only rarely in the feces of patients with paralysis (Kirby and Evans, 1955; Dalldorf, 1955; Johnsson, 1955c, Rhodes and Beale, 1957).

In recent years more direct and convincing evidence that Group-B strains can cause meningitis has been afforded by the recovery of virus from the spinal fluid of patients during the acute stage of illness. This has now been accomplished with strains of all 5 Group-B types: Type 1 (Girardi et al, 1957); Type 2 (Hummeler et al, 1954; Rhodes and Beale, 1957; Melnick, 1957); Type 3 (Gabinus et al, 1952, Gard, 1954, Girardi et al, 1957, Hummeler, 1957; McLean et al, 1957), Type 4 (Gard, 1954, Hummeler, 1957, Rhodes and Beale, 1957), and Type 5 (Curnen, 1957).

The clinical findings in patients with meningitis attributable to a Coxsackie-B virus provide no distinctive indication of the virus type responsible. Meningitis may occur independently or in association with pleurodynia The latter is more common in older children and adults and is possibly more likely in patients infected with B-1 and B-3 viruses (Johnsson, 1954; Rivadeneira et al, 1957). The onset may be sudden or gradual In approximately half of the cases illness is initiated by a prodromal phase of 2 to 6 days and is occasionally interrupted by a brief asymptomatic period. It is noteworthy that

viremia with Coxsackie virus B-2 was demonstrated recently with serum obtained from a patient with prodromal symptoms 5 days prior to the onset of clinical meningitis (Shelokov and Habel, 1957). Malaise, loss of appetite, nausea and abdominal pain are common early Fever may be present at the onset or develop subsequently in association with the appearance of headache, drowsiness, vomiting, stiffness or discomfort in the neck and the back, and sometimes focal or generalized myalgia The elevation of temperature ranges only occasionally above 40° C and lasts from 3 to 10 days with a mean of about 4 to 5 days On physical examination patients do not appear particularly ill and reveal few objective indications of disease. Hyperemia of the pharynx may be present during the acute stages Resistance of the neck or the back to anterior flexion is usually demonstrable but not extreme and rarely persists for more than a few days longer than the fever The Kernig and the Brudzinski signs can usually be elicited The deep and superficial reflexes remain normal Evidence of muscular weakness is equivocal or lacking Tremor, persistent muscular stiffness and skin rash are not characteristic findings The total and differential white blood cell counts are usually normal or only slightly elevated Leukopenia is not a feature of the disease Examination of the cerebrospinal fluid early in the acute phase of illness reveals an increase in the number of leukocytes which in most instances does not exceed 500 In patients infected with B-5 virus the total counts may range as high as 2,000 (Curnen, 1957) In the differential count mononuclear cells outnumber the polymorphonuclear cells, the latter, however, may initially account for 10 to 50 per cent of the total The protein content of the fluid is normal or only slightly elevated The amount of sugar present is also normal Except in very young infants with associated myocarditis, the course of illness is characteristically uncomplicated and terminates in complete recovery, although in adults fatigue and irritability may persist for as long as 2 months

It is now apparent that in addition to the Group-B strains, a number of Group-A viruses may also cause aseptic meningitis Russian workers have reported the recovery from the stools of paralyzed children of a so-called "Type 4 poliovirus" which experimentally induced paralysis and lesions in monkeys typical of poliomyelitis (Chumakov et al, 1956) Russian strains of this agent have been identified recently as Coxsackie virus A-7, and other strains recovered in this country have been

1957) There is some evidence that Coxsackie A-7 virus may also cause aseptic meningitis in man (Johnsson, 1955c, Johnsson et al, 1957, Habel et al, 1957, Kilbourne and Goldfield, 1956). In a "controlled" study Coxsackie A-9 strains were recovered 5 times more frequently in patients with aseptic meningitis than was expected (Habel et al, 1957), and in 2 cases the A-9 virus has been recovered from spinal fluid (Melnick, 1957) In South Africa, Group-A viruses of unspecified type were recovered from 7 patients with aseptic meningitis, in 3 instances from the spinal fluid (Gear et al., 1956).

A "new" Group-A virus not neutralized by antisera for Types 1 to 19 was encountered frequently in scattered outbreaks of aseptic meningitis which occurred in Italy during the summer of 1955 The virus was reported to have been recovered from spinal fluid in 53 instances. Respiratory or gastro-intestinal prodromata frequently preceded the meningeal syndrome Pleocytosis of the cerebrospinal fluid was unusual, total counts up to 6,000 were recorded (Archetti et al, 1957)

During the summer and the fall of 1956, outbreaks of aseptic meningitis frequently associated with a maculopapular rash were prevalent in Europe (Tyrrell and Snell, 1956, Rotem, 1957, Garnett et al, 1957, Boissard et al, 1957, McLean and Melnick, 1957, Tyrrell et al, 1957, Lennartz et al., 1957, Hennessen, 1956, Nihoul and Quersin-Thiry, 1957, Archetti et al, 1957, Rita et al, 1956, Baumann et al, 1957). The disease appears to be attributable to a virus which was recovered in a high proportion of cases from the feces and frequently also from spinal fluid This agent, which has properties of a Group-A Coxsackie virus, is neutralized by antisera against ECHO virus Type 9 A proposal has been made that this agent should properly be

classified as a new member of the Coxsackie-A group of viruses (Quersin-Thiry et al , 1957).

EPIDEMIC MYALGIA OR PLEURODYNIA (BORNHOLM DISEASE)

The first published descriptions of this disease came from Norway (Daae, 1872; Homann, 1872), although apparently outbreaks had been observed in 1856 and 1863 in Iceland (Finsen, 1874) Accounts of the same or similar disease called by a variety of names have since been recorded in different parts of the world. The monograph by Sylvest (1934) represents a classic description of this malady and has stimulated greater attention to it During the past 10 years Group-B Coxsackie viruses have been found in association with pleurodynia under circumstances which strongly indicate an etiologic relationship In patients infected with a single B-1 type of virus initially designated Conn-5, whose illnesses resembled pleurodynia (Curnen et al , 1949, Curnen, 1950, Weller et al , 1950, Kilbourne, 1950, Shaw et al , 1950), the clinical features corresponded generally to those encountered in the past (Sylvest, 1934) and in a typical outbreak which occurred in 1947 in Massachusetts (Finn et al , 1949) Cases of pleurodynia associated with the B-1 virus were also observed in 1952 in Japan (Yokota, 1955) and during 1956 in various areas of Great Britain (Medical Officer, Editorial, 1957). Strains of Coxsackie virus B-3 have been recovered from patients in several widely separate epidemics of pleurodynia (Gabinus et al., 1952, Lazarus et al , 1952, Huebner et al , 1953, Warin et al., 1953, Disney et al , 1953; Johnsson, 1955d; Derrick et al , 1956) The associations of pleurodynia with B-4 Coxsackie virus has been reported from South Africa (Patz et al , 1953; Wilkins et al., 1955) A laboratory worker accidentally infected with B-2 virus had clinical manifestations of both pleurodynia and aseptic meningitis (Curnen, 1950, 1952, Shaw et al., 1950). In numerous other studies of pleurodynia strains of Group-B virus have been recovered but not identified further. In outbreaks of aseptic meningitis associated with B-5 infection pleurodynia has not been noted There is no convincing evidence that Group-A Coxsackie viruses can cause this syndrome.

Clinical manifestations encountered in patients with pleurodynia associated with infection by a Group-B Coxsackie virus correspond to those described by Sylvest and more recently summarized in the report of a B-3 outbreak in Great Britain (Warin et al , 1953). Fever and pain, which are almost invariably present together, are usually abrupt in onset; occasionally, they may be preceded for several hours or days by malaise, anorexia and other vague prodromal symptoms The pain is most commonly thoracic, especially in adults, and is often accompanied by headache Pain in the chest, located on either side or substernally, may be excruciating but varies considerably in character and severity It is intensified by movement and may last from 2 days to almost 2 weeks Abdominal pain occurs in approximately half the cases, more commonly in children, and may be present in the absence of thoracic pain. This is frequently periumbilical or located on the right side more often than on the left Aches in the trunk or the extremities are sometimes noticed Stiffness of the neck or the back is an occasional complaint Nausea and vomiting are infrequent Cutaneous hyperesthesia is unusual. The elevation and the duration of fever correspond to those noted in patients with aseptic meningitis The course of acute illness is sometimes diphasic Tenderness which may be present over the site of pain, particularly in the abdomen, appears to be superficial suggesting localization in the muscular wall rather than in the viscera. In some instances swelling of a muscle is noted. In occasional patients with stiffness of the neck or the back the complaint is manifested by resistance to anterior flexion. The occurrence of associated meningitis in some cases of pleurodynia was recognized in the past (Lindberg, 1936; Gsell, 1949) but has now been firmly established in typical cases attributable to Group-B Coxsackie viruses. In one series of 262 cases of pleurodynia with B-3 infection the incidence of proved meningeal involvement was 2.6 per cent (Warin et al , 1953) The meningeal disease and the characteristics of the cerebrospinal fluid in these circumstances correspond to those seen in uncomplicated meningitis attributable to the same virus In pleurodynia the blood picture, urinalysis and other clini-

cal laboratory determinations are usually within the normal range. Orchitis appears sometimes as a complication of pleurodynia (Sylvest, 1934; Warin et al, 1953) The course of the disease is usually self-limited and terminates in complete recovery

Myocarditis of the Newborn

Recent reports indicate that Group-B Coxsackie viruses may induce intra-uterine or neonatal infection manifested in newborn infants by clinical and pathologic evidence of acute myocarditis

In 1952 during an epidemic of Bornholm disease in Johannesburg, South Africa, 10 babies with myocarditis were observed (Javett et al, 1956). Of these 6 developed circulatory collapse and died Strains of Coxsackie virus B-3 were recovered from the feces of 2 surviving infants Lesions resembling those caused by a Group-B Coxsackie virus were seen in suckling mice injected with suspensions of brain prepared from each of 2 babies who died

Of 3 infants with myocarditis observed during 1954 in a maternity home in Southern Rhodesia 1 died and 2 recovered (Montgomery et al, 1955) Coxsackie virus B-4 was found in specimens of feces from the baby who died and from 1 of the survivors Strains of this virus were also recovered from patients with Bornholm disease and meningo-encephalitis which were prevalent at the same time

In 1955 during an epidemic of "summer grippe" among adults in Amsterdam, 4 fatal cases of myocarditis in newborn infants were studied (van Creveld and de Jager, 1956, Verlinde et al, 1956) The clinical diagnosis of myocarditis was confirmed by electrocardiographic changes during life and the demonstration of acute interstitial myocarditis at autopsy Strains of B-4 Coxsackie virus were recovered from the heart muscle of each patient and in 1 instance from the brain

Two similar cases were encountered in Boston (Kibrick and Benirschke, 1956) Coxsackie virus B-3 was detected in a specimen of thoracic spinal cord from 1 infant delivered by cesarean section who died on the 7th day of life presumably as the result of infection contracted in utero. Recovery of a B-4 Coxsackie virus from another infant with fatal myocarditis was also mentioned

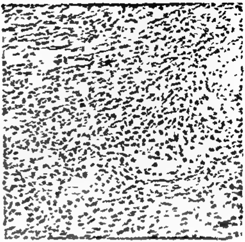

Fig 85 Myocarditis in a newborn infant caused by a B-4 Coxsackie virus (Creveld, S van, and de Jager, H, 1956, Myocarditis in newborns, caused by Coxsackie virus, clinical and pathological data, Ann Paediat *187*, 100-112)

The disease in newborn infants appears with sudden onset, in most instances within the first 8 or 9 days of life, sometimes a few hours or days after a brief episode of diarrhea and anorexia. Lethargy, grayish pallor and mild icterus are common features Tachycardia dyspnea and cyanosis, which may be present early in the course, indicate cardiac and respiratory embarrassment Either depression of body temperature or fever with elevations to 40° C may be encountered Cardiomegaly and hepatomegaly are characteristic features, the spleen may also be palpable Electrocardiograms show changes characteristic of myocarditis. Examination of the cerebrospinal fluid may reveal xanthochromic fluid, leukocytic pleocytosis and increased protein content.

In some cases the clinical course is biphasic, it may be rapidly fatal, even within an hour of apparent onset, or progress to complete recovery over a period of a week or longer Recovery of virus from the brain as well as from cardiac muscle in some cases, together with histologic evidence of lesions in brain and liver, indicate that myocarditis in the newborn is probably a feature of more generalized systemic disease comparable with that

classified as a new member of the Coxsackie-A group of viruses (Quersin-Thiry et al., 1957).

Epidemic Myalgia or Pleurodynia (Bornholm Disease)

The first published descriptions of this disease came from Norway (Daae, 1872; Homann, 1872), although apparently outbreaks had been observed in 1856 and 1863 in Iceland (Finsen, 1874). Accounts of the same or similar disease called by a variety of names have since been recorded in different parts of the world. The monograph by Sylvest (1934) represents a classic description of this malady and has stimulated greater attention to it During the past 10 years Group-B Coxsackie viruses have been found in association with pleurodynia under circumstances which strongly indicate an etiologic relationship. In patients infected with a single B-1 type of virus initially designated Conn-5, whose illnesses resembled pleurodynia (Curnen et al, 1949; Curnen, 1950, Weller et al., 1950; Kilbourne, 1950, Shaw et al, 1950), the clinical features corresponded generally to those encountered in the past (Sylvest, 1934) and in a typical outbreak which occurred in 1947 in Massachusetts (Finn et al, 1949) Cases of pleurodynia associated with the B-1 virus were also observed in 1952 in Japan (Yokota, 1955) and during 1956 in various areas of Great Britain (Medical Officer, Editorial, 1957). Strains of Coxsackie virus B-3 have been recovered from patients in several widely separate epidemics of pleurodynia (Gabinus et al, 1952, Lazarus et al., 1952, Huebner et al, 1953, Warin et al., 1953; Disney et al, 1953, Johnsson, 1955d, Derrick et al, 1956) The associations of pleurodynia with B-4 Coxsackie virus has been reported from South Africa (Patz et al., 1953, Wilkins et al, 1955) A laboratory worker accidentally infected with B-2 virus had clinical manifestations of both pleurodynia and aseptic meningitis (Curnen, 1950, 1952, Shaw et al, 1950) In numerous other studies of pleurodynia strains of Group-B virus have been recovered but not identified further. In outbreaks of aseptic meningitis associated with B-5 infection pleurodynia has not been noted. There is no convincing evidence that Group-A Coxsackie viruses can cause this syndrome.

Clinical manifestations encountered in patients with pleurodynia associated with infection by a Group-B Coxsackie virus correspond to those described by Sylvest and more recently summarized in the report of a B-3 outbreak in Great Britain (Warin et al, 1953). Fever and pain, which are almost invariably present together, are usually abrupt in onset; occasionally, they may be preceded for several hours or days by malaise, anorexia and other vague prodromal symptoms The pain is most commonly thoracic, especially in adults, and is often accompanied by headache Pain in the chest, located on either side or substernally, may be excruciating but varies considerably in character and severity It is intensified by movement and may last from 2 days to almost 2 weeks Abdominal pain occurs in approximately half the cases, more commonly in children, and may be present in the absence of thoracic pain This is frequently periumbilical or located on the right side more often than on the left. Aches in the trunk or the extremities are sometimes noticed Stiffness of the neck or the back is an occasional complaint. Nausea and vomiting are infrequent. Cutaneous hyperesthesia is unusual. The elevation and the duration of fever correspond to those noted in patients with aseptic meningitis. The course of acute illness is sometimes diphasic. Tenderness which may be present over the site of pain, particularly in the abdomen, appears to be superficial suggesting localization in the muscular wall rather than in the viscera In some instances swelling of a muscle is noted In occasional patients with stiffness of the neck or the back the complaint is manifested by resistance to anterior flexion. The occurrence of associated meningitis in some cases of pleurodynia was recognized in the past (Lindberg, 1936; Gsell, 1949) but has now been firmly established in typical cases attributable to Group-B Coxsackie viruses. In one series of 262 cases of pleurodynia with B-3 infection the incidence of proved meningeal involvement was 2 6 per cent (Warin et al, 1953). The meningeal disease and the characteristics of the cerebrospinal fluid in these circumstances correspond to those seen in uncomplicated meningitis attributable to the same virus. In pleurodynia the blood picture, urinalysis and other clini-

tion was demonstrated in a number of patients by Maryssael (1952) who used various electrical stimuli to analyze the nature of the lesion The early use of biopsy in the study of individuals infected with Coxsackie viruses (Dalldorf, 1949c) was abandoned to avoid danger to patients who might be infected with poliovirus

EXPERIMENTAL INFECTION, HOST RANGE

The experimental disease has been studied most thoroughly in mice, although similar responses occur in other animals, for example, hamsters, suckling ferrets and meriones The lesions in susceptible animals are the criteria used in identifying Coxsackie viruses and classifying them into Groups A and B This has proved to be reliable (Johnsson and Lundmark, 1955, Paola et al, 1952-53, Dalldorf, 1958) because even though the individual lesions are not strictly pathognomonic (see below) the pattern is distinctive There is no record of an individual Coxsackie virus of established type that causes lesions incompatible with the group to which it belongs The only confusion thus far has arisen from inocula that contained a mixture of viruses The combination of Group-A and -B lesions in an individual mouse should always suggest a search for a second virus

The pattern of the anatomic response varies with the route of inoculation Cerebral lesions, for example, are more frequent following Group-B infections if the animals have been injected intracerebrally (Gifford and Dalldorf, 1951) One lesion may predominate due to type and strain differences These properties may change with further adaptation, and some are known to depend on the tissue used for inoculation The pancreatotropism of certain Group-B viruses was lost following prolonged brain to brain transfer and intensified by the use of visceral and pancreatic suspensions (Dalldorf and Gifford, 1952) None of these differences seems to have seriously complicated diagnosis by histologic means The value of the distinction between the 2 groups rests largely on their significance in human disease Thus Bornholm disease and myocarditis neonatorum have only been associated with Group-B viruses, herpangina only with Group-A types

FIG 86 Focal necrosis of human muscle associated with a Group A Coxsackie virus infection (Gadeke, R, 1952, Vergleichende morphologische und chemische Muskelbefunde bei Säuglingsmausen und Meerschweinchen nach experimenteller Infektion mit Viren der Coxsackie-A-Gruppe, Klin Wchnschr *30*, 1040-1041)

As might be expected, the signs of disease in infant mice are also distinctive and will usually indicate the group to which the virus belongs

Group A Mice infected with Group-A viruses become helplessly paralyzed after a brief period of weakness Their extremities are leaden and immobile, and death soon follows, possibly from paralysis of the muscles of respiration Lépine et al (1952), who inoculated their mice intraperitoneally, noted herniation of the abdominal wall in many animals of the first 2 passages Presumably, as the strains became better adapted to mice, the local muscle lesion was submerged in the general degeneration of all skeletal muscles, and the hernias were no longer seen Those that survive for a week or more usually have whitish streaks in their muscles that are easily seen once the animal has been skinned Histologic examination of such animals will show a well-nigh universal degeneration or destruction of the striated muscles. The tongue and the heart are spared The most useful diagnostic features are the generalized myositis and the

seen in suckling mice infected with the same virus.

Cardiac complications of infection by Coxsackie viruses in older children and adults have not been reported frequently but may have been overlooked. Coxsackie B-2 virus was recovered from the feces of a 5-year-old boy in Ohio who had acute myocarditis and in whose blood a rising titer of neutralizing antibody against the virus was subsequently detected (McLean et al., 1957) Recently 2 cases of "benign pericarditis" were reported, 1 associated with a B-4 strain (Fletcher and Brennan, 1957) and another with a B-5 strain (Weinstein, 1957). Coxsackie virus B-5 was recovered from the stool of a 30-month-old boy with subacute myocarditis, and an associated increase in neutralizing and complement-fixing antibodies against this agent was detected (Varcasia and Castelli, 1957)

Herpangina

This clinical syndrome (Zahorsky, 1920, 1924), which occurs in the summer season and mainly affects children, has been found to be caused by any one of 6 different Group-A Coxsackie viruses, including Types 2, 4, 5, 6, 8 and 10 (Huebner et al, 1951, Parrott et al, 1951) The clinical and epidemiologic aspects of this disease have been reviewed recently (Parrott, 1957, Parrott and Cramblett, 1957)

The illness is initiated by a relatively abrupt onset of fever which affects 90 per cent of patients, ranges to a maximum of 40.5° C. and lasts from 1 to 4 days with a mean of about 2 days Anorexia and dysphagia are frequent symptoms Most patients over 2 years of age complain of sore throat, and about a third of them are afflicted with vomiting; fewer complain of headache or abdominal pain Convulsions associated with fever occur in about 5 per cent of cases Generalized myalgia is not a feature of the disease. Patients with herpangina do not appear very ill. The pharynx is usually hyperemic. Discrete and characteristic oral lesions may be seen They are commonly located on the anterior pillars of the fauces, less frequently on the palate, the uvula, the tonsils or the tongue and not on the gingival or the buccal mucosa. The early individual lesion appears as a grayish-white papule or vesicle about 1 to 2 mm. in diameter, surrounded by a red areola Within 2 or 3 days the areola becomes more intensely red, the vesicle enlarges and assumes the appearance of a shallow grayish ulcer which is rarely more than 5 mm. in diameter. Vesicles and ulcers may be present concomitantly and are usually evident during a period of 4 to 6 days after the onset of illness They range to 14 in number and average about 5 per patient. However, these lesions are not invariably detected during the disease Among closely associated persons infected by the same virus, typical vesicles or ulcers may be present in some and not in others Diarrhea, cough, rhinitis, otitis media, sinusitis and meningeal irritation are not characteristic features of herpangina The blood leukocyte counts are usually normal but may be somewhat elevated, especially if bacterial infection is also present However, pathogenic bacteria are associated with this syndrome only by coincidence The course of illness is usually benign and uncomplicated except for febrile convulsions in relatively few cases The findings of ulcers typical of herpangina adjacent to the vagina of a 7-year-old girl with the disease and the recovery of A-10 Coxsackie virus from these lesions, the throat and feces has been reported (Mitchell and Dempster, 1955). Recently, the occurrence of acute parotitis complicating herpangina in 4 patients infected by a Group-A Coxsackie virus was also noted (Howlett et al., 1957).

PATHOLOGIC PICTURE

The lesions of herpangina are characteristic, and the occasional localized muscle tumefaction in Bornholm disease may also be considered a distinctive manifestation of Coxsackie virus infection The only anatomic observations of fatal human disease are the myocarditis, at times associated with a degree of encephalitis and in 1 case with inflammatory changes in the spinal cord (Kilbrick and Benirschke, 1956) that have been found in fatal cases of myocarditis in newborn infants (Javett et al, 1956, Montgomery et al, 1955; van Creveld and de Jager, 1956, Verlinde et al, 1956).

Myositis was noted in biopsy samples from 2 human cases by Lépine et al (1952b) and by Vivell et al (1952) in a 6-year-old boy infected with an A-2 virus. Muscle degenera-

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absence of lesions elsewhere Mice infected intramuscularly with Theiler or encephalomyocarditis viruses, for example, also may show Zenker's degeneration of the muscles, but the lesions are limited to the injected extremity (Rustigian and Pappenheimer, 1949), or, if inoculation has been intracerebral, to the muscles of the spinal column In both cases encephalomyelitis also occurs. Group-A Coxsackie viruses affect the entire musculature and do not cause lesions in other organs. These findings and the selective susceptibility of newborn mice have thus far been associated only with Group-A infections.

The process begins within the muscle fibers with loss of definition, followed by hyalin degeneration, fragmentation and clumping (Gifford and Dalldorf, 1951; Godman et al., 1952). Aumonier (1953) found the sequence of early events to be loss of "H" and "Z" lines, followed by loss of "A" and "I" disks. swelling of the myofibrils and, finally, complete loss of internal structure Kausche and Hoffman-Berling (1952) found dense perinuclear zones composed in large part of nucleoprotein within 24 hours of infection The nuclei contained increased amounts of RNA These phenomena disappeared when degeneration became apparent in stained preparations

The Na-K ratio is reversed within 30 hours following infection (Gadeke and Waltenberger, 1952). Sharp losses of creatin and potassium occur (Gifford and Dalldorf, 1949; Gädeke, 1952a) and the excretion of myoglobin may be sufficiently large to cause nephrosis (Gadeke, 1952b). In a subsequent study, Albrecht and Gädeke (1956) illustrated the early histochemical changes Nucleic acid accumulations, in masses of granular or elongated form, were prominent These did not represent an actual increase in nucleic acid content but a mere rearrangement An actual increase in inorganic phosphates was demonstrated and ascribed to the blockade of the normal formation of energy carriers due to the loss of muscle function Several studies of muscle alkaline phosphatase have been reported (Albrecht and Sauthoff, 1954). The succinoxidase, anaerobic glycolysis, and combined glucose-6-phosphate and 6-phosphogluconate activity of muscle is not altered prior to morphologic change The glycolytic activity is lowered on the third day following inoculation, when the mice are paralyzed (Green, 1956)

Mice a few days older may survive, and in them repair replaces degeneration Myoblasts are conspicuous, as well as leukocytes, and

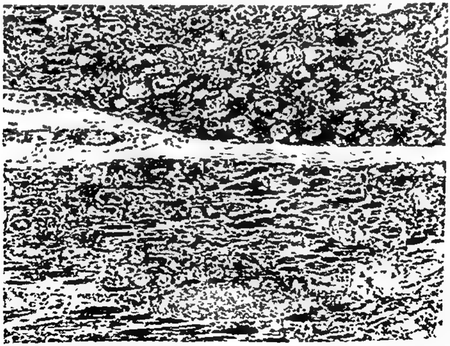

Fig. 87. Hyalin degeneration of striated muscle of an infant mouse infected with a Group A Coxsackie virus. The extreme cellularity is due to repair Extensive lesions of this kind are recognizable grossly.

TABLE 16 EFFECT OF THE ROUTE OF INOCULATION ON THE ISOLATION OF COXSACKIE VIRUSES

GROUP	NO ISOLATED BY ALL METHODS	NO ISOLATED BY IC ONLY	NO ISOLATED ONLY BY SC OR IP	NO ISOLATED BY EITHER METHOD
A	83	5	27	51
B	67	23	12	32

(Johnsson, 1955e)

regeneration advances rapidly. Resorption of degenerate muscle proceeds slowly, and evidence of damage may be found weeks later. [illegible]

Mice are susceptible to infection by the intracerebral, the intraperitoneal and the subcutaneous routes and to a much less extent (approximately 10 000 times) by the oral route (Kaplan and Melnick, 1951) Suckling mice 4 to 5 days old are fully susceptible to most Group-A viruses, and adapted strains may infect and paralyze many weanling mice and even older animals if sufficient virus is injected (Gifford and Dalldorf, 1949) Differences in susceptibility associated with route of inoculation are more striking during isolation Johnsson (1955e) has tabulated his experience (Table 16) It has been recommended that groups of mice be inoculated by each of 3 routes—intracerebral (IC), intraperitoneal (IP) and subcutaneous (SC)—and that further passages be made by all 3 routes if necessary (Dalldorf, 1958)

Certain Group-A viruses may induce myositis and paralysis in weaned mice, but serial transfers in such animals have not succeeded (Gifford and Dalldorf, 1949) On the other hand the representative strain of A-14 has been adapted to adult mice and induces in them lesions of the central nervous system similar to those that follow infections with the Lansing and the MEF strains of poliovirus (Dalldorf, 1957a) The adapted virus [illegible]

have been observed to cause similar lesions in adult mice on occasion Ward (1950) has mentioned neuronal necrosis and neuronophagia in the spinal cords of adult mice but did not identify the virus type

The A-14 virus that has been adapted to adult mice causes poliomyelitislike lesions in cynomolgous monkeys following intracerebral inoculation but not overt paralysis The original strains of A-7 also cause such lesions in monkeys (Dalldorf, 1957a), and an A-7 virus isolated by Chumokov et al. (1956) from paralytic cases of poliomyelitis in children frequently causes paralysis in monkeys as well This virus, identified by Chumokov as AB-IV, or poliovirus Type 4, has since been identified as Coxsackie virus A-7 (Johnsson and Lundmark, 1957, Habel and Loomis, 1957, Dalldorf, 1958) Habel and Loomis examined the brains and the spinal cords of 7 monkeys In 4, the lesions could not be distinguished from those caused by poliovirus In the remaining 3, the distribution was somewhat different The A-7 viruses studied thus far have not been pathogenic for adult mice, while the A-14 virus is and, indeed, the monkey and the mouse pathogenicity are associated to a degree

A number of strain and type differences occur among Group-A viruses in addition to those noted A-4, which has at times caused poliomyelitislike lesions in adult mice, is somewhat different in the responses of suckling mice (longer incubation period on primary isolation) and the low incidence of infection (antibodies) in man (Beeman and Huebner, 1952) The titer, in mice, is relatively low, a characteristic of many strains of A-7 and A-9, 11, 13, 15, 16, 17, 18 and 19 as well (Dalldorf, 1957) Only one Group-A type (2) has proved to be readily adapted to growth in embryonated hens' eggs This type includes a single strain that readily causes lesions of the striated muscles of chick embryos that are comparable with those seen in mice Type

FIG 88. Lesions of the spinal cord of an adult mouse following infection with the adult mouse adapted Coxsackie A-14 virus (*Top*) Distribution of the lesion which is largely limited to the anterior horns (*Bottom*) Destruction of motor neurones and their phagocytosis (Dalldorf, G, 1957, Neuropathogenicity of certain Group-A Coxsackie viruses, J. Exper. Med *106*, 69-76)

4 has a lesser ability to grow in chick cells Ten types of Group-A viruses were included in the study (Shaw, 1952) Godenne and Curnen (1952) tested 17 strains, including 16 different antigenic types for growth on the chorio-allantoic membrane. Evidence of multiplication was obtained only with 1 strain (Eastern 10) of Coxsackie Group A, type 10 virus

Cockroaches (*Periplanata americana*) were shown to remain infected with an A-4 Coxsackie virus for 15 days following feeding (Fischer and Syverton, 1951). In a later study it was reported that the gastro-intestinal tracts and salivary glands remained infectious for 20 days (Fischer and Syverton, 1957). The tropical rat mite (*Liponyssus bacoti*) also remained infectious for 2 weeks after feeding on infected mice but rarely transmitted infection to other mice or its progeny (Schwab, Allen and Sulkin, 1952). The virus used was also an A-4 Blowflies and houseflies were both found to harbor Coxsackie viruses for some days following inges-

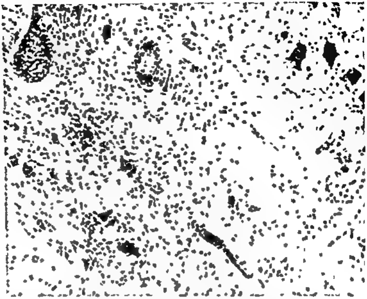

FIG 89 Poliomyelitislike lesion of the spinal cord of a monkey infected with a Coxsackie A-7 virus (Habel, K, and Loomis, L. N, 1957, Coxsackie A-7 virus and the Russian "Poliovirus Type 4," Proc Soc Exper. Biol & Med *95*, 597-605)

tion, but no evidence of virus multiplication was secured (Melnick and Penner, 1952)

A Group-A, Type-4 virus was also found to cause inapparent infections in wild cottontail rabbits and was once isolated from the blood of a naturally infected rabbit trapped in lowlands contaminated with sewage (O'Connor and Morris, 1955) Neutralizing antibodies for A-10 Coxsackie virus were found in nearly half of 40 swine sera and less frequently in rat and cow sera by Pohjanpelto and Vuopio (1956)

Group B In contrast with the inert helplessness of mice infected with Group-A viruses, Group-B mice exhibit tremors, spasticity and spastic paralysis * On dissection, gross evidence of muscle necrosis is uncommon, but changes in the interscapular fat pad may occur that are recognizable with the naked eye, as well as fat necrosis in the region of the pancreas Moreover, the brain may show softening

The degeneration of striated muscles following infection with Group-B viruses is characteristically focal and limited, and the changes in their Na and K content is less marked than following Group-A infections (Carcassi and Gioannini, 1956) The lesions most useful in diagnosis are degeneration of the brown fat (interscapular fat pad), encephalomalacia and, to a lesser degree, hepatitis, myocarditis, and necrosis of the acinar tissues of the pancreas The necrosis of the

ruses Sanz Ibañez, who found no muscle degenertaion in his mice, reported that the peripheral, muscle nerve fibers and motor plates showed nodular (rosary) degeneration (1951). The lesion of the fat pads was for a

The lesion is selective for the brown fat and begins as a degeneration of the cells, usually first on the surface of lobules and progressing to areas of frank necrosis with healing, regeneration and calcium depositions

The cerebral lesion, which occurs in 85 per cent of mice inoculated intracerebrally but in only a fourth of those inoculated intraperitoneally, begins as a patchy dissolution of the cerebral cortex followed by widespread

* A remarkable "zebroid lesion" formed by atro-

104)

FIG 90. Group B Coxsackie virus infection. (*Top*) An early stage of degeneration of the brown fat (*Bottom*) Typical appearance of the early changes in the cerebrum. These are the lesions most useful in identifying Group B infections in mice

liquefaction The lesion is easily recognized grossly The early changes are associated with the cortical blood vessels The necrosis occurs simultaneously in many foci, and encephalomalacia develops very quickly If, because of the age of the mouse, the animal survives, great cysts may be formed in the hemispheres Comparable changes were noted in the spinal cords of some mice by Pappenheimer et al (1950). Levaditi (1951) has discussed the

Lesions of the heart had been noted in many mice infected with the Powers strain (B-4) by Pappenheimer et al (1950). They were less frequently associated with other strains (largely B-1) and occurred in only 3 of 51 mice infected with various field strains of Group-B viruses (Gifford and Dalldorf, 1951). Melnick and Godman (1951) found myocardial necrosis in 3 of 35 4- to 5-day-old mice infected with a B-1 virus and approximately twice as often in animals inoculated the day of birth. The changes are a patchy necrosis followed by fragmentation and hyalin degeneration. The lesion may occur in any part of the myocardium Clusters of eosinophilic granules were found in certain muscle cells (Gifford and Dalldorf, 1951), and these have been the subject of a special investigation (Pappenheimer, 1952). The granules, in-

tensely fuchsinophilic, are from 500 to 600 mμ in diameter, roughly spherical, and were found by Pappenheimer in all the organs in which characteristic Coxsackie virus lesions occur. Pappenheimer referred to these inclusions as "F" granules In his studies, a B-1 virus was used (Conn-5 strain). They have been associated with at least one other

the former the only change was a slowing of the rhythm, but the Group-B mice also showed a lengthened PR interval and in some cases auriculoventricular block

The susceptibility of older mice is sharply increased if they are exposed continuously to cold (4° C) from shortly after inoculation In such animals, Boring et al. (1956) found the infectivity of the tissues increased 10-fold and observed lesions of heart, fat and pancreas It is interesting to note that the effect of cold is quite different from that of cortisone, which was early shown (Kilbourne and Horsfall, 1951) to transform benign into fatal infections in adult mice The effect of cortisone is largely directed to the heart and results in extensive myocardial lesions The cortisone does not influence virus multiplication, which proceeds equally in its absence, but rather acts synergistically (Kilbourne et al, 1956)

The appearance of hepatitis has been unpredictable Hepatitis leading to frank icterus and gastro-intestinal hemorrhage has been associated with some Group-B virus infections but inconstantly The lesions are usually less extensive and consist again of small areas of degeneration and even necrosis of parenchymal cells It is not difficult to distinguish between these lesions and those due to mouse hepato-encephalitis virus, since the latter develops more slowly and is surrounded by a fairly characteristic inflammatory reaction. The occurrence in the liver sinusoids of large refractile cells, possibly derived from the Kupffer cells, noted by Pappenheimer et al (1950), has not attracted the attention of other writers Some of the bodies may have been involuting megakaryocytes

A remarkable necrosis of the acinar epithelium of the mouse pancreas had been noted by Pappenheimer et al. (1950) in animals infected with several Group-B viruses Later, this lesion was studied in greater detail by Pappenheimer et al (1951). The process was remarkable in several respects The destruction was limited to the acinar structures, the islands of Langerhans and ducts being spared, adult mice were susceptible as well as suckling animals. In the older mice, none of the other Group-B lesions occurred The older mice characteristically survived and showed evidence of chronic pancreatic insufficiency, loss of weight, hypoproteinemia and at times anasarca

The discrepancy between these observations and those of Gifford and Dalldorf (1951) were resolved when it was learned that the pancreatotropism of the viruses used was irrevocably lost following prolonged brain-to-brain passage It was present in the same strain on reisolation from the human specimen and could be maintained by passage of visceral suspensions and intensified by pancreas transfers Pancreatic lesions have been rather frequently associated with certain strains and less so with others The method of maintaining the virus and strain differences apparently both play a part

The remarkable events that take place in the pancreas have been studied by means of electronmicrographs (Robertson, 1954). Vacuoles were found in the cytoplasm within 18 hours following infection. They coalesced until the cytoplasm was filled with them. In addition laminated bodies appeared as well as inclusions, sometimes as large as 5 mμ

Howes (1954) successfully titrated several Group-B strains in weaned mice, using pancreatic necrosis as the criterion of infection His method consisted of injecting trypan blue intraperitoneally 4 hours before the mice were killed. The dye made the pancreatic lesions sufficiently conspicuous to read grossly.

Gravid mice become increasingly more susceptible to infection with a pancreatotropic strain of B-1 virus during the last week of pregnancy. A Group-A virus did not have such an effect (Dalldorf and Gifford, 1954).

The effect of whole body radiation on young adult mice infected with the Powers (B-4) Coxsackie virus was an increase in the titer and the persistence of the virus Mortality was little affected (Cheever, 1953).

Coxsackie-B viruses rapidly multiplied in HeLa tumor masses transplanted to the peritoneal cavity of rats and, on serial passage through rat-HeLa tumors, developed increased capacity for destroying solid carcinomata in vivo (Suskind et al, 1957)

Fig. 92. Group B Coxsackie virus infection. (*Top*) An early stage of degeneration of the brown fat. (*Bottom*) Typical appearance of the early changes in the cerebrum. These are the lesions most useful in identifying Group B infections in mice.

liquefaction. The lesion is easily recognized grossly. The early changes are associated with the cortical blood vessels. The necrosis occurs simultaneously in many foci, and encephalomalacia develops very quickly. If, because of the age of the mouse, the animal survives, great cysts may be formed in the hemispheres. Comparable changes were noted in the spinal cords of some mice by Pappenheimer et al. (1950). Levaditi (1951) has discussed the mechanism of this "coelogenesis." Degeneration of motor neurons, including at times neuronophagia, has occasionally been seen in suckling hamsters inoculated intraperitoneally (Gifford and Dalldorf, 1951).

Lesions of the heart had been noted in many mice infected with the Powers strain (B-4) by Pappenheimer et al. (1950). They were less frequently associated with other strains (largely B-1) and occurred in only 3 of 51 mice infected with various field strains of Group-B viruses (Gifford and Dalldorf, 1951). Melnick and Godman (1951) found myocardial necrosis in 3 of 35 4- to 5-day-old mice infected with a B-1 virus and approximately twice as often in animals inoculated the day of birth. The changes are a patchy necrosis followed by fragmentation and hyalin degeneration. The lesion may occur in any part of the myocardium. Clusters of eosinophilic granules were found in certain muscle cells (Gifford and Dalldorf, 1951), and these have been the subject of a special investigation (Pappenheimer, 1952). The granules in-

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Marcolongo et al. (1956) compared newborn mice infected with Group-A and Group-B viruses by means of electrocardiograms. In the former the only change was a slowing of the rhythm, but the Group-B mice also showed a lengthened PR interval and in some cases auriculoventricular block.

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INFECTIONS WITH BOTH POLIOMYELITIS AND COXSACKIE VIRUSES

The first attempts to determine whether Coxsackie and polioviruses interfere were made with Group-A viruses (Howitt and Nichols, 1952; Melnick, 1950) No effect was demonstrated. Later it was learned that Group-B viruses exert a sparing effect on experimental poliomyelitis in mice (Dalldorf, 1951, Sulkin et al, 1953, Stanley, 1952, Domok, 1957) The Group-A viruses do not have such an effect—further evidence of the considerable biologic differences between the groups. Group-B viruses have also been shown to interfere with the survival of cells subsequently inoculated with poliovirus in vitro This was first demonstrated by Le Bouvier (1954) and has been confirmed by De Lustig and Brieux (1957) As might be expected from the mouse experiments, a Group-A virus had no such effect (Ledinko and Melnick, 1954).

The possibility that interference occurs in nature has been discussed The evidence consists of the frequent observation that paralytic poliomyelitis has occurred infrequently during outbreaks of Bornholm disease and evidence of an inverse relationship between the isolation of Group-B viruses and the incidence of paralytic disease (Dalldorf, 1955; Dalldorf and Albrecht, 1955). It is noteworthy that the opposite has been noted regarding Group-A viruses and paralytic poliomyelitis This was true of the patients from whom the original Coxsackie virus was isolated, both of whom were paralyzed It was true the following year of 2 small outbreaks in New York (Dalldorf, 1952) and of an unusually severe epidemic in Easton, Pa (Melnick et al, 1951) Poliomyelitis virus was isolated from 27 of 36 patients, and Coxsackie virus from 27 of 42 that were tested. In 24 instances type 1 virus was isolated. The association of the two was so intimate that the suggestion was made that the Coxsackie infection might well have been a predisposing factor of the paralysis Similar, if less striking examples, are commonplace The experience in the South African Institute for Medical Research (1955, 1956) is representative of the results in many laboratories where extensive investigations have been undertaken

In 1955 and 1956 Group-A viruses alone were isolated from patients with paralytic poliomyelitis Of 27 paralyzed patients studied in 1955, 23 yielded poliovirus, and 5 Group-A viruses as well. In 1956 polioviruses and Group-A viruses were associated in 17 patients, none of whom yielded a Group-B virus The greater association of poliovirus with an A rather than a B virus was significant statistically.

TABLE 17

	Coxsackie Virus Isolated	
Poliovirus	Group A	Group B
Not isolated	34	14
Isolated	17	0

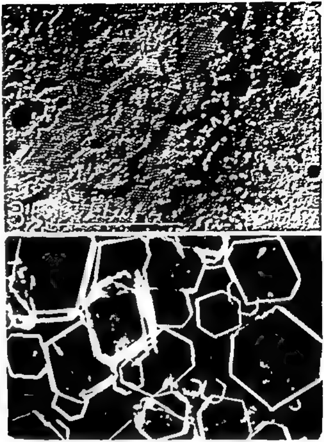
The probability of such a distribution occuring by chance = 0 0079

ETIOLOGY

Viruses of the Coxsackie group are relatively small and pass readily through bacteria-tight filters without significant loss in titer Results of filtration through gradocol membranes of known porosity indicate that the diameters of Group A, Types 1, 4 and 9, and Group B, Types 1 and 2, are about 20 to 29 mμ (Quigley, 1949, Himmelweit et al., 1950; Melnick et al, 1951, Melnick, 1955) This was true of virus contained in human feces, in the brain or the muscle of experimentally infected mice, and in infected tissue culture fluids The calculation of size is based on a factor of 0 64 multiplied by the size of the limiting pore diameter (i e, the A P D through which the virus just passes, or the average between the smallest A P D. fully passing the virus and the largest holding it back) (Black, 1958).

Ultracentrifugation in the partition cell of the analytical rotor, or through sucrose gradients in tubes in an angle rotor, yielded sedimentation constants of about 150 S and estimated diameters of about 25 to 30 mμ for the A-1, 2, 4 and 10 and B-1 and 2 strains studied (Melnick et al., 1951; Breese and Briefs, 1953) Direct examination of purified preparations of A-2, 4 and 10 in the analytical

FIG. 91. Coxsackie virus A-10 An electronmicrograph of a purified preparation (Breese, S S, Jr, and Briefs, A, 1953, Certain physical properties of a herpangina strain and a pleurodynia strain of Coxsackie virus, Proc. Soc. Exper. Biol. & Med 83, 119-122) The lower photograph is of the crystalline form of the same virus (Mattern, C. F. T., and DuBuy, H. G, 1956, Purification and crystallization of Coxsackie virus, Science 123, 1037-1038)



ultracentrifuge and in the electronmicroscope yielded values of 28mμ (see Fig 91) (Breese and Briefs, 1953; Mattern and DuBuy, 1956)

Coxsackie viruses are relatively stable Suspensions of virus may be preserved frozen at −70° or −20° C. for long periods without significant loss in titer Viral activity is also maintained when specimens of infected tissue are stored in 50 per cent glycerol or horse serum at room temperature for as long as 70 days, and in a refrigerator for over a year (Melnick and Ledinko, 1950) Like poliomyelitis viruses, these agents withstand exposure to a wide range of pH, i e, pH 2.3 to 9 4 for 1 day and 4 0 to 8 0 for 7 days (Robinson, 1950) Aqueous suspensions of certain strains *were not inactivated at 55° C. for 30 minutes* but were at 60° C. for the same period, temperatures 10° to 20° C. higher were required for inactivation when the virus was suspended in milk, cream, or ice cream (Kaplan and Melnick, 1952) Coxsackie viruses were not inactivated or not inhibited in vitro or in vivo by a variety of chemotherapeutic agents, including penicillin, streptomycin, chloramphenicol, oxytetracycline and viscosin Certain commonly used antiseptics, including ethanol (70%), Lysol (5%), Roccal (1%) and ether, also failed to inactivate the agents However, treatment with 0 1 N HCl or 0 3 formaldehyde, effected rapid inactivation (Melnick, 1951).

Group A, type 10 has been purified from suckling mice carcasses by a combination of salting out and ultracentrifugation procedures Upon storage at 4° C., the virus particles *form dodecahedral crystals about 100 mμ in* size (see Fig 91) The crystals have a titer of 10^{13} LD_{50} per cm^3. As this volume con-

tains about 4.6×10^{16} particles of 28 mμ diameter, the ratio of number of particles to number of infective units was about 4,600 to 1. A similarly large ratio of noninfectious to infectious particles could be calculated in the crude infected tissue suspensions (Mattern and DuBuy, 1956). About 4 per cent of the virus particle is made up of nucleic acid; the preliminary evidence suggests that it is of the ribose type

DIAGNOSIS

Diagnosis of infection by a Coxsackie virus may be suggested by clinical and epidemiologic manifestations, particularly of aseptic meningitis, pleurodynia, herpangina, or myocarditis occurring in the newborn However, confirmation must be obtained in the laboratory by the recovery of the virus from specimens of blood, cerebrospinal fluid, feces or oropharyngeal swabbings collected during the acute phase of illness and demonstration of a related increase in neutralizing antibodies against the virus in serum collected from patients during the acute and the convalescent stages of illness. Tissue culture technics have to a considerable extent supplemented but not supplanted the use of suckling mice for these purposes Rises in titer of complement-fixing antibodies against heterologous as well as homologous Coxsackie viruses occur in human serum following infection by these agents Hence, determinations by this technic are of limited diagnostic value It should be emphasized that demonstration of *infection* by a Coxsackie virus can be achieved by laboratory methods, but diagnosis of *disease* caused by one of these agents requires careful correlation of supporting clinical, epidemiologic and laboratory evidence

Differentiation of aseptic meningitis caused by a Coxsackie virus from mumps meningo-encephalitis, lymphocytic choriomeningitis, infection by ECHO viruses and other viral agents or by leptospira may be indicated by clinical and epidemiologic findings. Diagnosis of infection with these agents can usually be established by appropriate serologic tests and also in some instances by recovery and identification of the responsible agent, especially when obtained from the cerebrospinal fluid In view of the acute self-limited course of infection with Coxsackie virus in most instances and the paucity of associated neurologic signs, differentiation from bacterial meningitis, multiple sclerosis, or space-occupying intracranial lesions such as tumor, abscess, or hemorrhage, usually should not be difficult. Differentiation of Coxsackie virus infection from nonparalytic poliomyelitis in individual patients with aseptic meningitis does not appear to be possible on the basis of clinical findings alone, although persistence of spasm in isolated muscle groups is more suggestive of poliomyelitis. Although Coxsackie viruses have been recovered frequently from patients with a clinical picture of paralytic poliomyelitis, a causal relation of Coxsackie virus to paralytic disease is relatively uncommon and probably limited to only a few types, In most instances the association is probably coincidental Coxsackie and poliomyelitis viruses have been found together in the feces of individual patients with or without paralysis. On the basis of present knowledge, it appears reasonable for the clinician or the epidemiologist to consider and treat any acute febrile illness with associated paralysis as poliomyelitis until convincing evidence in support of an alternative diagnosis is demonstrated.

Epidemiologic evidence of seasonal and of local or regional prevalence is of value in the

be differentiated in individual patients from other causes of thoracic pain, particularly pneumonia, pleurisy and coronary thrombosis and from other causes of abdominal pain including acute appendicitis, peptic ulcer, disease of the gallbladder and acute pancreatitis. The superficial distribution of pain, the absence of deep abdominal or rectal tenderness, a relatively normal blood leukocyte count, and the absence of abnormal roentgenologic findings should aid in the diagnosis of infection by Coxsackie virus which can usually be verified by recovery of the virus and demonstration of an associated specific antibody response in the patient's serum The complication of orchitis in patients with pleurodynia or aseptic meningitis must be differentiated from mumps, a virus infection in which meningo-encephalitis often without parotitis may also be a manifestation

Diagnosis of herpangina is suggested by the acute self-limited course of the disease and the presence of typical faucial lesions The condition most likely to be confused with herpangina is infectious gingivostomatitis due to herpes simplex virus which may be associated with vesicular or ulcerative lesions in any part of the oral cavity, at mucocutaneous junctions, and in the skin Coxsackie virus infections are seasonal and may be epidemic, whereas herpes simplex infections are sporadic and without particular season distribution Coxsackie and herpes simplex viruses are both readily isolated in suckling mice and in tissue cultures. They can be identified and differentiated by various technics in the laboratory The oral manifestations of other conditions which may occasionally require differentiation from herpangina include the exanthems of acute exanthematous diseases and lesions associated with moniliasis, infectious mononucleosis, heavy metal poisoning, deficiency diseases and certain blood dyscrasias

Myocarditis in newborn infants must be differentiated from other forms of neonatal infection A diagnosis may be suggested by concurrent Coxsackie virus infection in the community and particularly in the mother at about the time of delivery The possibility of infection by a Coxsackie virus should be considered in any epidemic occurrence of severe disease in newborn infants and in newborn infants with signs of cardiorespiratory embarrassment

Because the clinical picture of infection by Coxsackie viruses is probably still incomplete, the possibility of infection by one of these agents should also be considered in cases of acute unexplained infections, particularly those affecting the neromuscular or cardiovascular systems and the pancreas, and especially when they occur in characteristic seasonal and epidemic distribution

It should be remembered that in a strict sense etiologic diagnosis may be difficult or complicated, since a feature of enteric virus infections is the tendency toward multiple infection Since it is probable that a number of enteric viruses have not yet been identified and may not be recognized by current technics, the possibility remains of the presence of undetected agents Moreover, a thorough search for the known viruses is laborious and hardly suitable for routine use A first principle should be that, once an agent has been isolated and identified, the original specimen be retested in the presence of specific antiserum for the agent recovered In this way additional viruses may not infrequently be found

The relative efficiency of isolations of Group-A viruses in suckling mice and monkey kidney cell cultures may be in the nature of 19 to 1 as judged by the prevalence of types in the past (Dalldorf, 1957) This obviously will vary from time to time with the proportion of cytopathogenic strains As a rule, a search for Coxsackie viruses cannot be considered to be satisfactory if mice are not inoculated, and it is well to inoculate 3 litters (randomized) by the subcutaneous, the intraperitoneal and the intracerebral routes The relative advantage of 2 routes of inoculation was determined by Johnsson (1955e) (cf Table 16). In the author's experience 3 routes afford further advantage

The Group-B viruses are much more suitable for isolation in tissue culture Monkey kidney and human amnion cells are superior in degree to HeLa cells (Table 18). At times Group-B viruses have been isolated more readily in tissue culture than in mice The reverse occurs less frequently. In the case of A-9, isolation in tissue culture is more efficient than in suckling mice. This was conspicuously true of the epidemic strains serologically related to ECHO 9 that occurred in Europe during 1955 and 1956 Many of these strains could not be directly isolated in mice, although they were, as a rule, pathogenic for mice after growth on monkey kidney cells

Methods that have been used successfully in the isolation, the grouping and the typing of Coxsackie viruses have been published (Dalldorf and Sickles, 1956) Grouping is possible from observation of the mice and histologic examination Typing of Group-B strains requires the use of the individual antisera The Group-A typing sera may be combined into two pools if their titers are satisfactory. The first represents Types 1, 4, 6, 7 and 9, and the second, Types 2, 3, 5, 8
[illegible]

TABLE 18. COXSACKIE VIRUSES

Coxsackie Virus		Cytopathogenicity			
Group	Type	Monkey Kidney	HeLa Cells	Uterine Plasma Clot	Other Characteristics
A	1	−	−	−	Occasional paralysis in 10 to 12 Gm mice (Gifford and Dalldorf, 1949, Dalldorf, 1957a)
	2	−	−	−	Two strains grown readily in eggs (Huebner et al 1950b, Shaw, 1952) Number of strains grown in embryonic chick suspended cell cultures (Shaw, 1952)
	3	−	−	−	
	4	−	−	−	Strain grown in suspended cell culture of minced embryonic mouse (Slater and Syverton, 1950) and embryonic chick tissue (Shaw, 1952)
	5	−	−	−	
	6	−	−	−	
	7	−	−	−	Lesions in CNS of monkey (Dalldorf, 1957a, Habel and Loomis, 1957)
	8	−	−	−	One strain multiplied on chorioallantoic membrane of embryonated eggs (Godenne, and Curnen, 1952)
	9	+	−	+	
	10	−	−	−	
	11	−	+	+	
	12	−	−	−	
	13	−	+	+	
	14	−	−	−	Lesions in CNS of adult mice and monkeys (Dalldorf, 1957a)
	15	−	+	+	
	16	−	−	−	
	17	−	−	−	
	18	−	+	+	
	19	−	−	−	
B	1	+	+	+*	Cytopathogenic for cultures of mouse interscapular fat pad tissue and mouse skeletal muscle (Stulberg et al, 1952, 1954) Growth in cultures of human embryonic brain and intestine and mature kidney tissue (Weller et al, 1953) Pancreatitis in adult mice (Pappenheimer et al, 1951) Cytoplasmic fuchsinophilic granules in suckling mouse tissue (Pappenheimer, 1952)
	2	+	±†	−	
	3	+	+	+*	Pancreatitis in adult mice (Dalldorf and Gifford, 1952)
	4	+	±†	−	Pancreatitis in adult mice (Dalldorf and Gifford, 1952)
	5	+	+	+*	Fuchsinophilic granules in liver (Dalldorf, 1956)

(See facing page for notes)

sackie viruses have been of minor degree and are taken into account in the assembling of the Group-A pools Typing sera have been prepared under the auspices of the Committee on Enteroviruses of the National Foundation for Infantile Paralysis

Etiologic diagnosis depends on isolation of the virus, since the number of types is too large to justify serologic tests unguided by knowledge of the type of virus that may be responsible In the presence of an epidemic, of course, this rule may be broken Infection should be confirmed by serologic tests, preferably neutralization tests in tissue culture or mice The complement-fixation reaction is of limited diagnostic value but may be used to advantage in typing This is especially true of Group-A viruses (Beeman et al, 1952, Contreras et al, 1952)

TREATMENT

No form of therapy is known which specifically affects infection induced by viruses of the Coxsackie group Various supportive and symptomatic measures may ameliorate certain features of the illnesses

EPIDEMIOLOGY

Viruses of the Coxsackie group have been encountered in all parts of the globe Isolations have been made mainly from human feces, pharyngeal swabbings, sewage and flies Widespread distribution is also indicated by the detection of antibodies in serum from individuals collected in different parts of the world and by the capacity of gamma globulin prepared from pooled human serum to neutralize all of the Coxsackie viruses thus far tested Evidence that the capacity of gamma globulin to neutralize Coxsackie viruses is an indication of previous specific antigenic experience is supported by the results of neutralization tests with serum collected from Eskimos in northern Alaska (Banker and Melnick, 1951), which revealed a population heavily exposed to A-4 and A-10, less so to A-1, and not at all to B-1 and B-2 In tests with specimens of feces which were collected in the same area, strains of Type 10 were isolated from each of 2 Eskimo children.

Information concerning the geographic and temporal distribution of individual types has become extensive. The earliest studies were suggestive Thus, in 1948, B-1 viruses were prevalent in Connecticut and Rhode Island (Curnen et al., 1949) as well as in New York (Dalldorf and Gifford, 1951) The same type was retrospectively proved to have been present in Massachusetts in 1947 (Weller et al., 1950) but thereafter was found infrequently for a number of years The striking prevalence of B-3 during 1951 in many parts of the world has been noted (Dalldorf, 1955) and has now been matched by the wave of infections due to the virus designated, at present, as ECHO, Type 9. These are examples of the pattern that has characterized many epidemics.

Most Coxsackie viruses have been recovered from specimens collected during the summer and the early fall Although this reflects to some extent the fact that these agents were first detected and later sought in the course of investigations on poliomyelitis, additional evidence has been obtained to indicate that Coxsackie viruses actually are encountered more frequently during the summer months. In epidemiologic surveys during 1949 and 1950, investigators (Huebner et al, 1950a, 1951, Cole et al., 1951) tested samples of feces collected at different times from individual inhabitants of a residential community near Washington, D C Isolations of virus were made almost exclusively in July, August and September.

Notes for Table 18

(Coxsackie viruses of Group B and A-9 produce round plaques on monkey kidney cells under agar, resembling those of the poliovirus [illegible]

[illegible] has slower and less marked

† B-2 and B-4 strains more erratic on HeLa cells than B-1, 3, 5

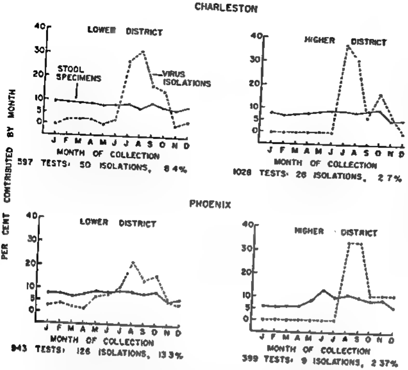


FIG. 92. Stool collections and enteric virus isolations. The cumulative score for each month during a 3-year period The specimens were collected from healthy children in Charleston, W. Va, and Phoenix, Ariz, during a nonepidemic period (Melnick, J. L, 1957, Special publication of the New York Acad. of Sc. *5*, 365-381)

In one study (Melnick and Ledinko, 1951) neutralizing antibodies to the local Coxsackie virus, A-4, were present in the spring in 90 per cent of the newborns but in only 15 per cent in the 6- to 12-month-old group The incidence was higher with increasing age reaching the adult level of 85 per cent in the 7- to 9- and 10- to 14-year-old age groups During the summer there was an increase in antibodies in all age groups except that of 7 years and over in which the adult level had already been reached Thus, in all children 6 years of age and under, 42 per cent were positive in May

economic group and 33 per cent for those in the lower group As in the case of

of 50 per cent dropped to 10 per cent in the 6- to 12-month age group, following which it progressively rose to reach 65 per cent in the 7- to 9-year age group This was of the same order as the adult level, and it was at this period that the neutralizing antibodies also reached the adult level. However, for the complement-fixing antibody there was a marked fall to 25 per cent positive in the 10- to 14-year age group, in contrast with the maintenance of the neutralizing antibody level. As with the neutralizing antibodies, an increase in complement-fixing antibodies took place during the summer in all age groups except in those 7 years and over In view of the fact that over 80 per cent of the children 7 years of age and

years and under, showed a 4-fold or greater drop in antibody titer during the summer In keeping with the maintenance of neutralizing antibody levels and decrease in complement-fixation antibody levels, it is worth noting that practically all children with complement-fixing antibodies had neutralizing antibodies as well.

An attempt was made to recover Coxsackie viruses from 860 patients in New Haven, Conn, who had acute febrile illnesses between March, 1950, and April, 1951. Forty-four strains were isolated, 36 of which were encountered in the period from July to October (Curnen and Godenne, 1952).

Coxsackie viruses have been recovered from

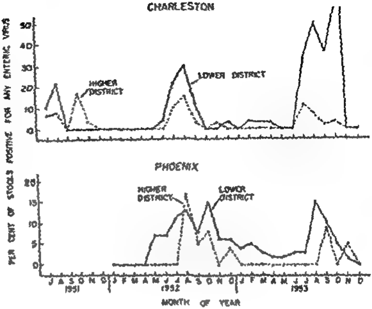


FIG. 93 The results of the Charleston-Phoenix study arranged to show the recurrent seasonal incidence of enteric virus excretion among normal children (Melnick, J L, 1957, Special publication of the New York Acad of Sc *5*, 365-381)

persons of both sexes and more frequently from children than from adults However, sufficient data are not yet available to determine accurately the incidence according to sex, race, or age-specific attack rates, this is particularly true of infections caused by different types.

Consecutive tests for several years for Coxsackie viruses in urban sewage and flies have been carried out in 6 states (Melnick et al, 1954a, b, Kelly et al, 1955, Kelly, 1957) In almost every urban area studied, virus appeared in some of the specimens collected in the summer and the fall Virus was detected in the community during the cold months but was not widely disseminated, for it was *only* a rare winter specimen that yielded virus. Even though present each summer, the frequency of virus recovery during the months of peak occurrence varied in the same community from year to year, ranging from 10 to 100 per cent The recovery of Coxsackie viruses was more regular from sewage than from flies in the same area In Muskegon, Mich, where in 1 year Melnick et al (1954) isolated 34 Coxsackie strains from sewage, 26 belonged to a single antigenic type, A-1. In Topeka, Kans, Coxsackie viruses were recovered through the summer and the fall of 1 year, but A-5 was present in the summer, and it was replaced by A-6 in the fall Thus the long persistence of virus for several months in a residential area, or its reappearance after a short period, may be due to the serial dissemination of new types of virus rather than to recurring waves of infection by the original virus In Albany, N Y, Kelly (1957) observed similar changing annual patterns In 1953, A-5 and A-6 were frequently isolated, but 1 year later the predominant types were A-4, A-8 and B-2 In these respects all of the recognized enteric viruses behave similarly

In a longitudinal study recently reported (Melnick, 1957b, Honig et al, 1956) approximately equal numbers of stools were collected each month from normal children living under contrasting environments in Charleston, W Va, and Phoenix, Ariz As shown in Figures 92 and 93, almost all of the virus isolations were made in the summer and the fall, except from the lower socio-economic district of Phoenix where viruses were recovered throughout a larger part of the year Figure 93 illustrates the recurrent seasonal incidence of enteric virus excretion among normal children

The frequency of virus excretion in the lower socio-economic district of each city was 3 to 6 times as great as in the middle to upper middle-class districts with good environmental sanitation If the combined figures are examined (Table 19) of 1,540 specimens tested in the lower districts, 176 (11 4%) were posi-

TABLE 19. PER CENT DISTRIBUTION OF ENTERIC VIRUSES ISOLATED FROM HEALTHY CHILDREN IN POPULATIONS OF CONTRASTING SOCIO-ECONOMIC LEVEL DURING A NONEPIDEMIC PERIOD (1951–53) *

Population Group	Number of Specimens Tested	Per Cent Yielding Viruses			
		Polioviruses	Coxsackie Viruses	ECHO Viruses	All Enteric Viruses
Charleston, W Va					
Lower	597	2.3	2.3	3.7	8.4
Upper	1028	0.5	1.5	0.8	2.7
Phoenix, Ariz					
Lower	943	3.0	2.0	8.3	13.3
Upper	399	1.0	1.0	0.3	2.3
Total					
Lower	1540	2.8	2.1	6.6	11.4
Upper	1427	0.6	1.3	0.6	2.6

* From Melnick (1957b)

tive, whereas in the upper districts only 2.6 per cent (37 of 1,427 specimens tested) yielded virus Among the 213 viruses isolated in monkey kidney cultures, 52 per cent were ECHO viruses, 24 per cent were polioviruses, and 24 per cent were Coxsackie viruses

Similarities in the epidemiology of poliomyelitis and infection by Coxsackie viruses may be briefly summarized Both poliomyelitis and Coxsackie viruses induce in man infections associated with specific antibody responses In the human host both agents may be carried for some time and may cause either recognizable disease or inapparent infection. They are encountered most frequently at the same time of year Both agents may be recovered not only from man but also from flies and sewage and at times may be found together in the same specimen When both kinds of virus are found together in a patient with or without paralysis, it is not possible to determine precisely the extent to which each agent contributes to the observed [illegible]

may [illegible] above).

CONTROL MEASURES

Specific measures to control infection by Coxsackie viruses have not been devised These agents may be distributed by means of the oropharyngeal secretions and the feces of infected persons, many of whom remain undetected Flies and cockroaches may harbor and transport Coxsackie viruses, but whether they are of importance in the transmission of Coxsackie-virus diseases from person to person has not yet been determined Because of the unusual stability of Coxsackie viruses at ordinary temperatures and their resistance to alcohol and other substances commonly used as antiseptic solutions, decontamination of objects which cannot be boiled or autoclaved may not be achieved by conventional methods However, Coxsackie viruses can be inactivated rapidly by 0.1 N hydrochloric acid or 0.3 per cent formaldehyde No reports have appeared on the efficacy of vaccines in prophylaxis

REFERENCES

Albrecht, W, and Gadeke, R., 1956, Weitere chemische, histochemische und histologische Untersuchungen uber Veranderungen in Geweben von Saugungen nach Coxsackie-Virus-Infektion, Ztschr f. Naturforsch *11b*, 241-247.

Albrecht, W., and Sauthoff, R, 1954, Über das Verhalten der alkalischen Phosphatase im Muskel der mit Coxsackie-Virus infizierten Säuglingsmaus, Ztschr f Naturforsch, *9b*, 340-342.

Archetti, I, Cited in Dalldorf, G, 1957, Brief review of Coxsackie viruses in 1956, Ann New York Acad. Sc *67*, 209-211

Archetti, I, and Felici, A, 1956, L'agente etiologico di una forma di meningite asettica rescontrata in forma epidemica, Riv Ist sieroterap ital *31*, 265

Archetti, I., Felici, A., Rossi, F, and Fua, C, 1957, Ricerche sulla definizione eziologica della meningonevrassite marchigiana, condotte durante l'ondata epidemica estivo-autunnale 1955, Policlinico (sez prat) *64*, 224-234

Aumonier, F J., 1952, Structural changes in the skeletal muscles of suckling mice following infection with a Coxsackie virus of the "A" group, J Roy Micro Soc *72*, 218-222

Banker, D D, and Melnick, J L, 1951, Isolation of Coxsackie virus (C virus) from North Alaskan Eskimoes, Am J Hyg *54*, 383-390

Baumann, T, Barben, M, Marti, R, Hassler, A., and Krech, U., 1957, Erkrankungen durch ECHO-virus typ 9, Schweiz med Wchnschr *87*, 307-315

Bayer, P, and Gear, J., 1955, Virus meningo-encephalitis in South Africa A study of the cases admitted to the Johannesburg Fever Hospital South African J Lab & Clin Med *1*, 22-35

Beeman, E A, and Huebner, R J., 1952, Evaluation of serological methods for demonstrating antibody responses to Group A Coxsackie (herpangina) viruses, J Immunol *68*, 663-672

Beeman, E A, Huebner, R J., and Cole, R M., 1952, Studies of Coxsackie viruses Laboratory aspects of Group A viruses Am J Hyg *55*, 83-107

Black, F L, 1958, Relationship between virus particle size and filterability through gradocol membranes Virology In press

Boissard, G P B, Stokes, L J, Macrae, A D, and MacCallum, F O, 1957, Isolation of viruses related to ECHO virus Type 9 from outbreaks of aseptic meningitis, Lancet *1*, 500

Boring, W D, ZuRhein, G M., and Walker, D L, 1956, Factors influencing host-virus interactions II Alteration of Coxsackie virus infection in adult mice by cold Proc Soc Exper Biol & Med *93*, 273-277

Breese, S S, Jr, and Briefs, A, 1953, Certain physical properties of a herpangina strain and a pleurodynia strain of Coxsackie virus, Proc Soc Exper Biol & Med *83*, 119-122

Carcassi, U, and Gioannini, P., 1956, Aspetti del metabolismo muscolare nell'infezione sperimentale del topino con virus Coxsackie, Riv Ist sieroterap ital *31*, 277-281

Cheever, F S, 1953, Multiplication of Coxsackie virus in adult mice exposed to roentgen radiation, J Immunol *71*, 431-435

Chumakov, M P., Voroshilova, M K, Zhevandrova, V I, Mironova, L L, Itzelis, F I, and Robinson, I A, New methods of detection and identification of Poliomyelitis virus, 1956 Problems of Virol *1*, 16

Cole, R M, Bell, J A., Beeman, E A., and Huebner, R J., 1951, Studies of Coxsackie viruses Ob- [illegible]

[illegible] 1181-1185

Contreras, G, Barnett, V. H, and Melnick, J L, 1952, Identification of Coxsackie viruses by immunological methods and their classification into 16 antigenically distinct types J Immunol. *69*, 395-414

Creveld, S van, and de Jager, H., 1956, Myocarditis in newborns, caused by Coxsackie virus Clinical and pathological data Ann paediat *187*, 100-112

Curnen, E C, 1950, Human disease associated with the Coxsackie viruses, Bull New York Acad. Med *26*, 335-342

Curnen, E C, and Melnick, J L, 1952, The Coxsackie viruses *in* Poliomyelitis Papers and Discussions Presented at the Second International Poliomyelitis Conference, p 423, Philadelphia, Lippincott

Curnen, E C, Jr., 1957 Personal communication

Curnen, E C., and Godenne, M O, 1952, Propagation of Coxsackie Virus on the Chorioallantoic Membrane of Embryonated Eggs, Proc Soc Exper Biol & Med *81*, 81-85

Curnen, E C, Shaw, E W, and Melnick, J. L, 1949, Disease resembling nonparalytic poliomyelitis associated with a virus pathogenic for infant mice, J.A.M.A *141*, 894-901

Daae, A, 1872, Epidemi i drangedal af akut muskelreumatisme udbredt ved smitte Norsk mag laegevidensk *2*, 409-413

Dalldorf, G, 1949a, The Coxsackie group of viruses, Science *110*, 594

———, 1949b, The etiology of poliomyelitis, New York J Med *49*, 2053-2054

———, 1949c, Suggested studies of poliomyelitis, New York J Med *49*, 1330

———, 1950, The Coxsackie viruses, Bull New York Acad Med *26*, 329-335

———, 1951, The sparing effect of Coxsackie virus infection on experimental poliomyelitis, J Exper Med *94*, 65-71

———, 1952, From Clostridium welchii to the Coxsackie viruses changing microbiology, J Mt Sinai Hosp *19*, 396-410

———, 1955, The Coxsackie viruses, Ann Rev Microbiol *9*, 277-296

———, 1956, Problems related to the Coxsackie viruses Presented at Conference on Tests for Diagnosis and Determination of Immune Status in Poliomyelitis, New York, May 7-8, 1956, held under the auspices of the National Foundation for Infantile Paralysis.

———, 1957a, Neuropathogenicity of certain Group-A Coxsackie viruses J Exper Med *106*, 69-76

———, 1957b, Brief review of Coxsackie viruses in 1956, Ann New York Acad Sc *67*, 209-211

———, 1958, The Coxsackie viruses—1957 *in* Poliomyelitis Papers and Discussions Presented at Fourth International Poliomyelitis Conference, Philadelphia, Lippincott

Dalldorf, G., and Albrecht, R, 1955, Chronologic association of poliomyelitis and Coxsackie virus infections, Proc Nat Acad Sc *41*, 978-982

Dalldorf, G, and Gifford, R, 1951, Clinical and epidemiologic observations of Coxsackie-virus infection, New England J Med *244*, 868-873

Dalldorf, G, and Gifford, R, 1952, Adaptation of Group B Coxsackie virus to adult mouse pancreas, J Exper Med 96, 491-497

———, 1954, Susceptibility of gravid mice to Coxsackie virus infection, J Exper Med 99, 21-27

Dalldorf, G, and Sickles, G M, 1948, An unidentified, filtrable agent isolated from the feces of children with paralysis, Science 108, 61-62

———, 1956, The Coxsackie viruses in Diagnostic Procedures for Virus and Rickettsial Diseases, ed 2, pp 153-168, New York, Am Pub Health Assoc

Dalldorf, G, Sickles, G M, Plager, H, and Gifford, R, 1949, A virus recovered from the feces of "poliomyelitis" patients pathogenic for suckling mice, J Exper Med 89, 567-582

DePaola, D, Goes, P de, Burno-Lôbo, M, Vasconcellos, J V, and Travassos, J, 1952-1953, Ocorrência de virus Coxsackie no Rio de Janeiro, An Microbiol Rio de Janeiro 2, 89-99

Derrick, E H, Pope, J H, Sinnamon, C N, Stanley, N F, Dorman, D C, and Ponsford, J, 1956, Coxsackie infection in Queensland, M J Australia 43, 961-966

Disney, M E, Howard, E M, Wood, B S B, and Findlay, G M, 1953, Bornholm disease in children, Brit M J 1, 1351-1354

Dömok, I, 1957, Studies on the interaction between Coxsackie and poliomyelitis viruses I. Simultaneous infection with B 1 Coxsackie and Lansing poliomyelitis viruses in mice of different ages Acta Microb, Hungary 4, 183-195

Findlay, G M, and Howard, E M, 1950, Coxsackie viruses and Bornholm disease, Brit M J 1, 1233-1236

Finn, J J, Weller, T. H, and Morgan, H. R, 1949, Epidemic pleurodynia clinical and etiologic studies based on one hundred and fourteen cases, Arch Int Med 83, 305-321

Finsen, J, 1874, Iagttagelser angaaende Sygdomsforholdene i Island, pp 145-151, Copenhagen, Reitzels

Fischer, R G, and Syverton, J T, 1951, The cockroach as an experimental vector of Coxsackie virus, Am. J. Trop Med 31, 238-242.

———, 1957, Distribution of Coxsackie virus in experimentally infected cockroaches, *Periplaneta americana*, Proc Soc Exper Biol & Med 95, 284-287

Fletcher, E, and Brennan, C F, 1957, Cardiac complications of Coxsackie-virus infection, Lancet 1, 913-915

Gabinus, O, Gard, S, Johnsson, T, and Poldre, A, 1952, Studies on the etiology of epidemic pleurodynia (Bornholm disease) I Clinical and virological observations Arch ges Virusforsch 5, 1-13

Gädeke, R, 1952a, Kidney lesions in suckling mice following Group A Coxsackie virus infection, A.M.A Arch. Path 54, 276-280

———, 1952b, Vergleichende morphologische und chemische Muskelbefunde bei Säuglingsmäusen und Meerschweinchen nach experimenteller Infektion mit Viren der Coxsackie-A-Gruppe, Klin Wchnschr. 30, 1040-1041

Gädeke, R, and Waltenberger, H, 1952, Histologische, histochemische und chemische Untersuchungen an der Skeletmuskulature von Säuglingsmäusen und saugenden Meerschweinchen zur Frage der Pathogenität von Coxsackie-A-Viren, Ztschr Naturforsch 7b, 524-531

Gard, S, 1954, The etiology of acute aseptic meningitis (Wallgren), Acta paediat 43, 54-64

Gard, S., and Johnsson, T, 1952, Studies of Coxsackie viruses, Acta path et microbiol scandinav, (Suppl 93), pp 332-339

Garnett, D G, Burlingham, A, and Zwanenberg, D van, 1957, An outbreak of aseptic meningitis of virus origin in East Suffolk, Lancet 1, 500-502

Gear, J. H S, 1955, Reports of Official Delegates, Union of South Africa in Poliomyelitis. Papers and Discussions Presented at the Third International Poliomyelitis Conference, pp 60-61, Philadelphia, Lippincott

Gear, J, Measroch, V, and Prinsloo, F R, 1956, The medical and public health importance of the Coxsackie viruses, South African M J 30, 806-810

Gifford, R, and Dalldorf, G, 1949, Creatinine, potassium, and virus content of the muscles following infection with "Coxsackie virus," Proc. Soc Exper Biol & Med 71, 589-592

———, 1951, The morbid anatomy of experimental Coxsackie virus infection, Am J Path 27, 1047-1063

Girardi, A J, Hummeler, K, and Olshin, I, 1957, Studies on the routine laboratory diagnosis of Coxsackie Group B virus infections I The application of tissue culture procedures J Lab & Clin Med 50, 526-533

Godenne, M O, and Curnen, E C, 1952, Propagation of Coxsackie virus on the chorioallantoic membrane of embryonated eggs, Proc Soc Exper Biol & Med 81, 81-85

Godman, G C, Bunting, H, and Melnick, J L, 1952a, The histopathology of Coxsackie virus infection in mice I Morphologic observations with four different viral types Am J Path 28, 223-257

Green, M R, 1956, Carbohydrate metabolism of mouse muscle in relation to infection with Coxsackie virus, Group A, J Exper Zool 133, 481-504

Gsell, O, 1949, Meningitis myalgica Meningitis serosa bei Myalgia epidemica Schweiz med Wchnschr 79, 241-244

Habel, K, and Loomis, L N, 1957, Coxsackie A 7 virus and the Russian "Poliovirus Type 4," Proc Soc. Exper Biol & Med 95, 597-605

Habel, K, Silverberg, R J, and Shelokov, A, 1957, Isolation of enteric viruses from cases of aseptic meningitis, Ann New York Acad Sc 67, 223-229

Hennessen, W, 1956, Über das epidemische Auftreten einer bisher unbekanntes Virus-meningitis und ihren Erreger, Deutsche med Wchnschr 81, 2085-2090

Hummelweit, F, Findlay, G M, and Howard, E M, 1950, The size of Coxsackie viruses as estimated by filtration through gradocol membranes, Brit J Exper. Path 31, 809-812

Homann, C, 1872, Om en i Kragerø Laegedistrikt herskende smitsom Febersygdom, Norsk mag laegevidensk *2*, 542-555

Honig, E I, Melnick, J L, Isacson, P., Parr, R, Myers, I L, and Walton, M, 1956, An epidemiological study of enteric virus infections, J Exper Med *103*, 247-262

Howes, D W, 1954, Intravital staining in titrations of Group B Coxsackie viruses in weaned mice, Nature, London *173*, 270-271

Howitt, B F, and Nichols, V J, 1952, Inoculation of cynomolgus monkeys with Coxsackie virus alone, combined, or with poliomyelitis virus, J Immunol *68*, 599 608

Howlett, J G, Somlo, F, and Kalz, F, 1957, A new syndrome of parotitis with herpangina caused by the Coxsackie virus, Canad M A J *77*, 5-7

Hsiung, G D, and Melnick, J L, 1957a, Morphologic characteristics of plaques produced on monkey kidney monolayer cultures by enteric viruses, J Immunol *78*, 128-138

———, 1957b, Comparative susceptibility of kidney cells from different monkey species to enteric viruses, J Immunol *78*, 137-146

Huebner, R J, Armstrong, C, Beeman, E A, and Cole, R M, 1950a, Studies of Coxsackie viruses. Preliminary report on occurrence of Coxsackie virus in a southern Maryland community J A M A *144*, 609-613

Huebner, R J, Cole, R M, Beeman, E A, Bell, J A, and Peers, J H, 1951, Herpangina etiological studies of a specific infectious disease, J A M A *145*, 628-633

Huebner, R J, Ransom, S E, and Beeman, E A, 1950b, Studies of Coxsackie virus Adaptation of a strain to chick embryo Pub Health Rep *65*, 803-806

Huebner, R J, Risser, J A., Bell, J A, Beeman, E A, Beigelman, P M., and Strong, J C, 1953, Epidemic pleurodynia in Texas a study of 22 cases, New England J Med *248*, 267-274

Hummeler, K, 1957, Viruses in search of disease Discussion part I Ann New York Acad Sc *67*, 248-249

Hummeler, K, Kirk, D, and Ostapiak, M, 1954, Aseptic meningitis caused by Coxsackie virus with isolation of virus from cerebrospinal fluid, J A M A *156*, 676-679

Javett, S N, Heymann, S, Mundel, B, Pepler, W J, Lurie, H I, Gear, J, Measroch, V, and Kirsch, Z, 1956, Myocarditis in the newborn infant A study of an outbreak associated with Coxsackie Group B virus infection in a maternity home in Johannesburg J Pediat *48*, 1-22

Johnsson, T, 1954 Studies on the etiology of Bornholm disease (epidemic pleurodynia) II Epidemiological observations Arch ges Virusforsch *5*, 401-412

———, 1955a, Occurrence of Coxsackie virus infections in an epidemic of poliomyelitis, Arch ges Virusforsch *6*, 216-232

———, 1955b, A non-typable cytopathic agent as the probable cause of a family outbreak of aseptic meningitis, Arch ges Virusforsch, *6*, 223-241

———, 1955c, The occurrence of Coxsackie virus in a hospital material of paralytic poliomyelitis, aseptic meningitis and a miscellaneous group of diseases, Arch ges Virus forsch *6*, 250-261

———, 1955d, A virological, clinical and epidemiological study of Coxsackie virus infections, Bohuslaningens AB-Uddevalla, 30 pp

———, 1955e, A virological and clinical study of infections with Coxsackie virus, Bohuslaningens AB-Uddevalla, 40 pp

Johnsson, T, Jonsson, B, Lycke, E, and Wictorin, B, 1957, Studies of an epidemic of aseptic meningitis associated with Coxsackie and ECHO viruses I Virological observations Arch ges. Virusforsch *7*, 384-393

Johnsson, T., and Lundmark, C., 1955, A histopathological study of Coxsackie virus infections in mice, Arch ges Virusforsch *6*, 262-281

———, 1957, Identification of the "fourth type" of poliomyelitis virus (correspondence), Lancet *1*, 1148-1149

Kaplan, A S, and Melnick, J L, 1951, Oral administration of Coxsackie viruses to newborn and adult mice, Proc Soc Exper Biol & Med *76*, 312-315

———, 1952, Effect of milk and cream on the thermal inactivation of human poliomyelitis virus, Am J Pub Health *42*, 525-534

Kausche, G A, and Hoffman-Berling, H, 1952, Die Nucleinsäureverteilung in mit Coxsackie-Virus beimpften Mausemuskel, Ztschr Naturforsch *6b*, 518-524

Kelly, S, 1957, Enteric virus isolations from sewage, Acta med scandinav *159*, 63-70

Kelly, S M, Clark, M E, and Coleman, M B, 1955, Demonstration of infectious agents in sewage, Am J Pub Health *45*, 1438-1446

Kibrick, S., and Benirschke, K, 1956, Acute aseptic [illegible]

[illegible], 381-384

Kilbourne, E D, and Goldfield, M, 1956, Coxsackie viruses and "virus-like" diseases of the adult A three-year study in a contagious disease hospital Am J Med *21*, 175-183

Kilbourne, E D, and Horsfall, F L, Jr, 1951, Lethal infection with Coxsackie virus of adult mice given cortisone, Proc Soc Exper Biol & Med *77*, 135-138

Kilbourne, E D, Wilson, C B, and Perrier, D, 1956, The induction of gross myocardial lesions by a Coxsackie (pleurodynia) virus and cortisone, J [illegible]

[illegible]

Lazarus, A S, Johnston, E A., and Galbraith, J E., 1952, An outbreak of epidemic pleurodynia, with special reference to the laboratory diagnosis of Coxsackie virus infections, Am J Pub Health *42*, 20-26

LeBouvier, G L, 1954, Interference and cell protection by poliomyelitis virus in tissue culture, Nature, London 174, 649-651

Ledinko, N., and Melnick, J. L, 1954, Interference between poliomyelitis viruses in tissue culture, J Exper Med 100, 247-267.

Lennartz, H, Maass, G, and Kersling, G, 1957, Zur Ätiologie der abakteriellen Meningitis Ergebnisse einer virologischen Untersuchung der Meningitisepidemien im Sommer und Herbst 1956, Klin Wchnschr 35, 327-334

Lépine, P, Chaumont, L, and Blusson, J, 1952a, Sensibilite du merion aux virus Coxsackie, Ann Inst Pasteur 82, 643-650

Lépine, P, Desse, G, and Sautter, V, 1952b, Biopsies musculaires avec examen histologique et isolement du virus Coxsackie chez l'homme atteint de myalgie epidémique (Maladie de Bornholm) Bull Acad nat méd 136, 66-68

Lépine, P, Sautter, V, and Reine, L, 1952c, Hernies abdominales chez les souriceaux inocules avec le virus Coxsackie, Ann Inst Pasteur 82, 251-254

Levaditi, C, 1951, La "coelogenese" nevraxique du virus Coxsackie, Ann Inst Pasteur 81, 260-274

Lindberg, G, 1936, Myalgia epidemica im Kindesalter, Acta paediat 19, 1-49

Lustig, E S de, 1956, Interference entre les virus de Coxsackie et de poliomyelite sur les cultures de cellules HeLa, Compt rend Soc biol 15, 2017-2018

Lustig, E S de, and Brieux, S, 1957, Interference between Coxsackie virus B 4 and poliomyelitis virus in HeLa strain (Abst) Tissue Culture Assn Program of 8th Annual Meeting, Univ of Maryland School of Medicine, Baltimore, April 16-17, 1957.

McLean, D M, Croft, C C, Prince, J T, and Heckman, [redacted] E, 1957, Coxsackie and ECHO infections in Ohio during 1956, Ohio M J 53, 907-909.

McLean, D M, and Melnick, J L, 1957, Association of mouse pathogenic strain of ECHO virus Type 9 with aseptic meningitis, Proc Soc Exper Biol & Med 94, 656-660

Marcolonga, F, Carcassi, U, and Tuveri, A, 1956 Alterazioni elettrocardiografiche sperimentali in topini inoculati con virus Coxsackie (A 4 [redacted] 1), Riv Ist sieroterap ital 31, 282-286

Maryssael, L, 1952, La maladie de Bornholm Diagnostic differentiel avec la poliomyélite La formule des maladies de Bornholm Concours Méd 74, 453-459, 1065-1068, 2501-2502

Mattern, C F T, and Dubuy, H G, 1956, Purification and crystallization of Coxsackie virus, Science 123, 1037-1038

Medical Officer-Editorial, 1957, Bornholm disease, [illegible]

356

———, 1951, Poliomyelitis and poliomyelitis-like viruses of man and animals, Ann Rev Microbiol 5, 309-332

———, 1957a, Problems associated with viral identification and classification in 1956, Ann New York Acad Sc 67, 363-382.

———, 1957b, ECHO viruses, Special Publication of the New York Academy of Sciences, Vol. 5, pp 365-381

Melnick, J L, Emmons, J, Coffey, J H, and Schoof, H, 1954a, Seasonal distribution of Coxsackie viruses in urban sewage and flies, Am J Hyg 59, 164-184

Melnick, J L, Emmons, J Opton, E M, and Coffey, J H, 1954b, Coxsackie viruses from sewage Methodology including an evaluation of the grab sample and gauze pad collection procedures Am J Hyg 59, 185-195

Melnick, J L, and Godman, G C, 1951, Pathogenesis of Coxsackie virus infection Multiplication of virus and evolution of the muscle lesion in mice J Exper Med 93, 247-266

Melnick, J L, and Kaplan, A S, 1950, Dual antibody response to Coxsackie and poliomyelitis viruses in patients with paralytic poliomyelitis, Proc Soc Exper Biol & Med, 74, 812-815

Melnick, J. L, Kaplan, A S, Zabin, E, Contreras, G, Larkum, N. W, 1951, An epidemic of paralytic poliomyelitis characterized by dual infections with poliomyelitis and Coxsackie viruses, J Exper Med 94, 471-492.

Melnick, J L, and Ledinko, N, 1950, Immunological reaction of the Coxsackie viruses, I Neutralization test technic and application J Exper Med 92, 463-482

———, 1951, Social serology antibody levels in a normal young population during an epidemic of poliomyelitis, Am J Hyg 54, 354-382

———, 1953, Development of neutralizing antibodies against the three types of poliomyelitis virus during an epidemic period The ratio of inapparent infection to clinical poliomyelitis Am J Hyg 58, 207-222

M[illegible] [redacted] and Kraft,

Melnick, J L, and Penner, L R, 1952, The survival of poliomyelitis and Coxsackie viruses following their ingestion by flies, J Exper Med 96, 255-271

Melnick, J L, Rhian, M, Warren, J, and Breese, S S, Jr, 1951, The size of Coxsackie viruses and Lansing poliomyelitis virus determined by sedimentation and ultrafiltration, J Immunol 67, 151-162

Melnick, J L, Shaw, E W, and Curnen, E C, 1949, A virus isolated from patients diagnosed as non-paralytic poliomyelitis or aseptic meningitis, Proc Soc Exper Biol & Med 71, 344-349

Mitchell, S C., and Dempster, G, 1955, The finding of genital lesions in a case of Coxsackie virus infection, Canad M A J 72, 117-119.

Montgomery, J, Gear, J., Prinsloo, F R, Kahn, M., and Kirsch, Z G, 1955, Myocarditis of the newborn An outbreak in a maternity home in Southern Rhodesia associated with Coxsackie Group-B virus infection South African M J 29, 608-612

Nihoul, E, and Quersin-Thiry, L, 1957, A new clinical entity? Lancet *1*, 269-270

O'Connor, J R, and Morris, J. A, 1955, Recovery of Texas-1 type Coxsackie virus from blood of wild rabbit and from sewage contaminating rabbit's feeding ground, Am J Hyg *61*, 314-320

Pappenheimer, A M, 1952, Fuchsinophile granules in the tissues of mice infected with the Connecticut-5 strain of Coxsackie virus, J Exper Med *95*, 251-258

Pappenheimer, A M, Daniels, J B, Cheever, F S, and Weller, T H, 1950, Lesions caused in suckling mice by certain viruses isolated from cases of so called non-paralytic poliomyelitis and of pleurodynia, J Exper Med *92*, 169-190

Pappenheimer, A M, Kunz, L J, and Richardson, S, 1951, Passage of Coxsackie virus (Connecticut-5 strain) in adult mice with production of pancreatic disease, J Exper Med *94*, 45-64

Parrott, R H, 1957, The clinical importance of Group A Coxsackie viruses, Ann New York Acad Sc *67*, 230-240

Parrott, R H, and Cramblett, H G, 1957, Nonbacterial infections affecting the nasopharynx *in* Symposium on Respiratory Disorders, p 115, Pediatric Clinics of North America, Philadelphia, Saunders

Parrott, R H, Huebner, R J, McCullough, N B, Wolf, S I, and Naiden, E, 1955, The hospital as a factor in assessing the occurrence of Coxsackie viruses in various illness groups I Children from the outpatient department and various hospital wards Pediatrics *15*, 255-263

Patz, I M, Measroch, V, and Gear, J, 1953, Bornholm disease, pleurodynia or epidemic myalgia, outbreak in the Transvaal associated with Coxsackie virus infection, South African M J *27*, 397-402

Platt, H, 1956, A study of the pathological changes produced in young mice by the virus of foot and-mouth disease, J Path & Bact *72*, 299-312

Pohjanpelto, P, and Vuopio, P, 1956, Coxsackie antibodies in domestic animals and Norway rats, Ann med exper et biol Fenniae *34*, 390-392

[illegible]

Quigley, J J, 1949, Ultrafiltration and ultracentrifugation studies of Coxsackie virus, Proc Soc Exper Biol & Med *72*, 434-435

Ramos-Alvarez, M, and Sabin, A B, 1954, Characteristics of poliomyelitis and other enteric viruses recovered in tissue culture from healthy American children, Proc Soc Exper Biol & Med *87*, 655-661

Rhodes, A J, and Beale, A J, 1957, Aseptic meningitis evidence for the etiologic role of Coxsackie B and "orphan" viruses, Ann New York Acad Sc *67*, 212-222

Rhodes, A J, Clark, E M, Wood, W, Shimada, F T, Silverthorne, N, McKendry, J B J, Dun-
[illegible]

Rita, G, Russi, M, Fischer-Fantuzzi, L, 1956, Isolamento di virus Coxsackie da focolai epidemici marchigiani di meningite asettica acuta, Riv Ist sieroterap ital *31*, 266-272.

Rivadeneira, J C, Robbins, F C, Crea, M, and Eiben, R. M, 1957, The distribution of Group B Coxsackie viruses in a children's institution, Pediatrics *20*, 468-476

Robertson, J S, 1954, The pancreatic lesion in adult mice infected with a strain of pleurodynia virus I Electron microscopical observations Australian J Exper Biol & M Sc *32*, 393-409

Robinson, L K, 1950, Effect of heat and of pH on strains of Coxsackie virus, Proc Soc Exper Biol & Med *75*, 580-582

Rotem, C E, 1957, Meningitis of virus origin, Lancet *1*, 502-504

Rowe, W P, 1953, Propagation of Group A Coxsackie viruses in denervated adult mouse muscle, Science *117*, 710

Rubin, H, Lehan, P H, Doto, I L, Chin, T D Y, Heeren, R H, Johnson, O, Wenner, H A, and Furcolow, M L, 1958, Epidemic infection with Coxsackie virus Group B, type 5 Clinical and epidemiologic aspects New England J Med *258*, 255-263

Rustigian, R, and Pappenheimer, A M, 1949, Myositis in mice following intramuscular injection of viruses of the mouse encephalomyelitis group and of certain other neurotropic viruses, J Exper Med *89*, 69-92

Sanz Ibañez, J, 1951, Estudios sobre el comportamiento de las cepas A y B del virus Coxsackie, Trab Inst Cajal Invest Biol *43*, 165-186

Schwab, M, Allen, R, and Sulkin, S E, 1952, The tropical rat mite (*Liponyssus bacoti*) as an experimental vector of Coxsackie virus, Am J Trop Med *1*, 982-986

Shaw, E W, Melnick, J L, and Curnen, E C, 1950, Infection of laboratory workers with Coxsackie viruses, Ann Int Med *33*, 32-40

Shaw, M, 1952, Cultivation of Coxsackie virus in embryonated eggs and in chick tissue cultures, Proc Soc Exper Biol & Med *79*, 718-720

———, 1956, Adaptation of viruses to eggs, *in* Annual Report of the Division of Laboratories and Research, New York State Department of Health, Albany, pp 6-7

Shelokov, A, and Habel, K, 1957, Viremia in Coxsackie B meningitis, Proc Soc Exper Biol & Med *94*, 782-784

Shwartzman, G, and Aronson, S M, 1954, Participation of brown fat in pathogenesis of experimental poliomyelitis of monkeys, Proc Soc Exper Biol & Med *86*, 767-771

Slater, E A, and Syverton, J T, 1950, The cultivation of Coxsackie virus, Proc Soc Exper Biol & Med *74*, 509-510

South African Institute for Medical Research, 1955, 1956 Annual Report, pp 54-57, 49-51

Stanley, N F, 1952, Attempts to demonstrate interference between Coxsackie and poliomyelitis viruses in mice and monkeys, Proc Soc Exper Biol & Med *81*, 430-433

Stanley, N F, Dorman, D C, and Ponsford, J, 1953, A hitherto undescribed group of Coxsackie viruses associated with an outbreak of encephalitis, Australian J Exper. Biol & M Sc *31*, 31-40

Stulberg, C S, Schapira, R, and Eidam, C R, 1952, Virus growth in tissue culture fibroblasts II Coxsackie virus (Group B) in cultures of mouse fat tissue Proc Soc Exper Biol & Med *81*, 642-646

———, 1954, Comparative characteristics of two Coxsackie viruses in tissue culture, J Immunol *72*, 107-111

Sulkin, ■ E, Wallis, C, and Murphy, T. P, Jr, 1953, Mixed infections with Coxsackie and Lansing poliomyelitis viruses in mice, Proc Soc Exper Biol & Med *84*, 184-189

Suskind, R G, Huebner, R J, Rowe, W P, and Love, R, 1957, Viral agents oncolytic for human tumors in heterologous host Oncolytic effect of Coxsackie B viruses Proc Soc Exper Biol. & Med *94*, 309-318

Sylvest, ■, 1934, Epidemic Myalgia Bornholm Disease, Copenhagen, Levin & Munksgaard, 155 pp

Syverton, J T, McLean, D M, Martins da Silva, M, Doany, H B, Cooney, M, and Kleinman, H, 1957, Outbreak of aseptic meningitis caused by Coxsackie B 5 virus laboratory, clinical, and epidemiologic study, J.A.M.A *164*, 2015-2019

Tyrrell, D A J., Lane, R R, and Snell, B, 1957, Further studies of an epidemic of exanthem associated with aseptic meningitis, Quart J Med, in press

Tyrrell, D A J, and Snell, B, 1956, Recovery of a virus from cases of an epidemic exanthem associated with meningitis, Lancet *2*, 1028-1029

Varcasia, E, and Castelli, L, 1957, Su di uncaso di myocardite da Coxsackie B *5*, Rendic Inst. super san *20*, 831-841

Verlinde, J D, and Ting, L K, 1954, Experimental infection of suckling-ferrets with viruses of the Coxsackie group isolated from cases of infantile gastroenteritis, Antonie v Leeuwenhoek *20*, 181-184

Verlinde, J. D., van Tongeren, H. A. E, and Kret, A, 1956, Myocarditis in newborns due to Group B Coxsackie virus virus studies, Ann paediat *187*, 1-2, 113-118

Vivell, O, Gädeke, R, Roland, D, and Sievers, K, 1952, Klinische, virologische und pathologisch-anatomische Beobachtungen bei Coxsackie-Virus-Infektionen, Deutsche med Wchnschr *77*, 983-937

Ward, R, 1950, Some recent advances in poliomyelitis, J Pediat. *37*, 263-270

Warin, J F, Davies, J, ■ M, Sanders, F K, and Vizoso, A. D, 1953, Oxford epidemic of Bornholm disease, 1951, Brit M J *1*, 1345-1351

Webb, C H and Wolfe S G 1951, Three day fever an acute febrile disease of childhood (further observations), Am J Dis Child *80*, 245-253

Weller, T H, Enders, J F, Buckingham, M, and Finn, J. J., Jr., 1950, The etiology of epidemic pleurodynia a study of two viruses isolated from a typical outbreak, J Immunol *65*, 337-346

Weller, T H, Robbins, F C, Stoddard, M B, and Florentino, G L, 1953, Propagation of Coxsackie viruses in cultures of human tissues, J Immunol *71*, 92-97

Weinstein, S B, 1957, Acute benign pericarditis associated with Coxsackie Group B, Type 5, New England J Med *257*, 265-267

Wilkens, A J W, Kotze, D. M, Melvin, J, Gear, J, Prinsloo, F R, and Kirsch, Z, 1955, Meningo-encephalitis due to Coxsackie B virus in Southern Rhodesia, South Africa Med J *29*, 25-28

Yokota, T, 1955, Isolation of B Group Coxsackie virus from an outbreak of epidemic myalgia in Kawasaki in the summer of 1952 Personal communication

Zahorsky, J, 1920, Herpetic sore throat, South M. J *13*, 871-872

———, 1924, Herpangina (A specific infectious disease), Arch Pediat *41*, 181-184

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26

The ECHO Virus Group

INTRODUCTION

The ECHO (enteric cytopathogenic human orphan) viruses comprise a group of agents brought together chiefly because they infect the human intestinal tract and because they can be isolated only in certain tissue cultures Although only recently recognized, certain types are already known to cause aseptic meningitis with or without rash, febrile illnesses with or without rash, and diarrheal diseases in infants and children during the summer months. Twenty-four antigenic types have been identified thus far, and additional types are in the process of classification

HISTORY AND STATUS OF ECHO GROUP

The refinement of tissue culture methods and the expansion of their use into the field of virology led to the isolation of large numbers of hitherto unknown viruses that ordinarily fail to produce disease in laboratory animals It early became apparent that these

with the aseptic meningitis syndrome (Robbins et al, *1951*; *Steigmann* et al, *1953*; Melnick, 1954) and that multiple types exist (Melnick, 1954, Ramos-Alvarez and Sabin, 1954) As more investigators entered the field, more and more of these untypable agents were recognized. Because their relationship to human disease was unknown, and because they failed to produce illness in laboratory animals, including infant mice, they were called "orphan" viruses or human enteric viruses, a name later changed to *Enteric Cytopathogenic Human Orphan* viruses or ECHO viruses (Committee on the ECHO Viruses, 1955) A co-operative study by Melnick,

duce disease in laboratory animals, including newborn mice

During the past decade, a large number of hitherto unknown viruses have been found in the intestinal tract of man by use of newborn mice and tissue cultures for their isolation These viruses include the 19 group A and 5

many investigators (Johnsson, 1957; McLean and Melnick, 1957, Quersin-Thiry et al, 1957, Boissard et al, 1957; and others) that

a large proportion of epidemic strains recovered in Europe in 1956 produced paralysis in newborn mice after inoculation of the large amounts of virus contained in undiluted tissue culture fluid. *The paralysis was found to be* the result of muscle lesions indistinguishable from those produced by the Coxsackie A viruses However, unlike the classic Coxsackie A viruses, the original human specimens *containing the ECHO 9 viruses quite regularly* failed to produce paralysis, and even after passage in tissue culture there was great variation in the number of strains that acquired the capacity to produce paralysis with large

property of ECHO [illegible] viruses (Eggers and Sabin, *1958) indicated* great variation among different strains, with some strains (like the original prototype) the virus does not even multiply in newborn mice, while with others even 3 TCD_{50} may result in inapparent multiplication, and very large doses are required to produce paralysis. Serial passages in mice can select more paralytogenic virus particles; therefore, occasionally it might be possible to isolate certain strains by passage in mice as was accomplished by Gear and Dalldorf (personal communication). Since most other ECHO viruses have not been studied in a similar manner, it is not known how many others may possess variants similar to those of ECHO 9.

Among the 19 established Coxsackie A viruses there is only one type (A 9) that in its naturally occurring state is cytopathogenic for monkey kidney tissue cultures—the *optimum medium for the ECHO viruses—and* newborn mice are in most instances not sufficiently susceptible to permit its isolation directly from human material (Melnick, 1955, Honig et al, 1956, Habel et al, 1957). Thus, the question has arisen whether ECHO 9 virus should be transferred to the Coxsackie A group because some of its variants produce paralysis in newborn mice or Coxsackie A 9 virus should be transferred to the ECHO group because most of the naturally occurring strains possess the predominant characteristics of the ECHO viruses

It is obvious that no single property can be used to classify viruses, and especially when that property is absent in the majority of naturally occurring virus particles Not all naturally occurring viruses that are pathogenic only for newborn mice (e g., the dengue, the sandfly fever or the Sindbis viruses) or that produce paralysis with muscle lesions (e g., some of the encephalitis viruses) can be grouped with the Coxsackie viruses, nor can *all variants of viruses (e g, dengue) capable* of producing paralysis with lesions in the anterior horn cells of the spinal cord of monkeys be grouped with the poliomyelitis viruses Yet it is becoming increasingly apparent that *some variants of the Coxsackie viruses,* notably A 7 and A 14, as well as some of the ECHO viruses, can produce neuronal lesions in monkeys and that some variants of the polioviruses may lack the capacity of producing *either paralysis or neuronal lesions in* monkeys and chimpanzees In the Coxsackie B group there is also a certain proportion of naturally occurring strains that lack the

apparent that many properties, such as approximately similar size, certain cellular af-

myelitis viruses to warrant their inclusion in a single family of human *enteroviruses* (*Committee on the Enteroviruses, 1957*). The adenoviruses, herpes simplex and perhaps also the mumps and the influenza viruses, although affecting predominantly the respiratory and the upper alimentary tracts, may occasionally *be found or even multiply in the* intestinal tract. However, their size and other properties serve to distinguish them from the true enteroviruses The size of many of the viruses now in the Coxsackie and the ECHO *groups still remains to be determined.* On this basis at least one of the viruses now in the ECHO group, i e, ECHO 10, probably will form a distinct group, for different antigenic variants of this virus have been found by *gradocol membrane* filtration to have a size of 60 to 90 mμ (compared with 12 to 20 mμ by similar methods of measurement and calculation for the polioviruses and many of the ECHO and the Coxsackie viruses) and to be implicated in respiratory as well as enteric infections (Sabin, 1957a) Furthermore, mouse pathogenic variants producing lesions comparable with those of Coxsackie [illegible] viruses have been segregated from some of the strains (Dalldorf, 1957a; Sabin, 1957a), and at least one of these variants has been *found to multiply* in chick embryos (Dalldorf, 1957a). Furth

in the Coxsackie and the ECHO groups will ultimately provide the basis of a more definitive classification

It was first postulated (Committee on the ECHO Viruses, 1955) that whenever an ECHO virus was established as the etiologic agent of a clinically distinct disease, it would be removed from the ECHO group However, subsequent experiences have demonstrated that thus far the ECHO viruses have been established as etiologic agents only of clinical syndromes which may be caused by a variety of bacteria or viruses For example, ever since their first isolation and recognition, a number of different enterovirus types have been shown to be associated with, and responsible for, aseptic meningitis. However, because this is a syndrome rather than a clinically distinct disease, it will have to be designated in a particular patient as *aseptic meningitis due to ECHO type 6*, or whatever other virus type happens to be involved The same is true of the febrile illnesses with exanthem, or of the diarrheal infections in which ECHO viruses have been incriminated.

CLINICAL PICTURE

The incidence of clinically recognized disease produced by ECHO viruses varies with the type and the strain of virus in a manner comparable with various types and strains of poliovirus The clinical manifestations resulting from infection with certain types of ECHO virus are now based partly on observations during epidemics in which one type of ECHO virus predominated, as in certain outbreaks of the aseptic meningitis syndrome, febrile illnesses with exanthem, and infantile diarrhea, or on controlled studies of summer diarrheal diseases in early childhood The clinical manifestations associated with infection by different members of the enterovirus family are listed in Table 20 For establishment of etiologic association it was necessary to show a much higher prevalence of these viruses among patients with the disease than among noncontact healthy individuals of the same age and socio-economic status living in the same area at the same time as the patients It was also necessary to establish in individual patients the absence of concurrent infection with other agents already known to cause the same clinical syndrome Additional evidence of the role of ECHO viruses in the clinical

TABLE 20 CLINICAL MANIFESTATIONS ASSOCIATED WITH INFECTION BY ENTEROVIRUSES

Polioviruses

1 Paralysis (complete to slight muscle weakness)
2 Aseptic meningitis
3 Undifferentiated febrile illness, particularly during the summer

Coxsackie Viruses, Group A

1 Herpangina (Types 2, 4, 5, 6, 8, 10)
2 Undifferentiated febrile illness, particularly during the summer
3 Aseptic meningitis (Types A 7, A 9)
4 Febrile illness with rash (Type A 9)

Coxsackie Viruses, Group B

1 Aseptic meningitis
2 Pleurodynia
3 Undifferentiated febrile illness with pharyngitis
4 Myocarditis or encephalomyocarditis during neonatal period and early childhood
5 Mild paralysis (?) or encephalitis

ECHO Viruses

1 Aseptic meningitis (Types 2, 3, 4, 5, 6, 9, 14, 16, 21)
2 Febrile illness with rash (Types 4, 9, 16 and probably others)
3 Undifferentiated febrile illness
4 Mild paralysis (?) (Types 6, 9) or encephalitis (Type 9)
5 Summer diarrhea of infants and children (Type 18 and others)

manifestations to be described here was obtained by demonstrating antibody development during the course of the illness The frequent presence of the virus in the cerebrospinal fluid, particularly in ECHO 9 infections, provided further evidence for an etiologic association with the aseptic meningitis syndrome For the present it seems best not to generalize but to describe separately the clinical picture observed in association with infection by individual ECHO viruses

ECHO 4 Two outbreaks are on record. (1) In Marshalltown, Iowa, and vicinity in 1955 (Lehan et al, 1957, Chin et al, 1957) the clinical manifestations consisted predominantly of the aseptic meningitis syndrome and some associated minor illnesses but without exanthem, and (2) in Sweden in 1956 (Johnsson, 1957) in which an exanthem was

observed in some of the patients. In the Marshalltown outbreak, the onset was characteristically abrupt with fever and severe headache, frequently localized in the retrobulbar area. Disturbances of the alimentary tract in the form of nausea, vomiting or diarrhea were recorded in about 70 per cent of the patients and sore throat in about 40 per cent. Irritability was common, and pain in the muscles of the lower extremities was a symptom in about 40 per cent. All patients in the aseptic meningitis group complained of stiffness of the neck or the back or both, and this could be confirmed by physical examination; however, none exhibited either the Kernig or the Brudzinski signs. There were no abnormal reflexes. Minor transient weakness of one leg was noted in 2 of the 53 patients with the aseptic meningitis syndrome, but it was not indicated whether these might have been the patients from whom both ECHO 4 and polioviruses were recovered (4 of 21—Chin et al., 1957).

The fever was low grade (99° to 102° F.) and lasted 3 to 5 days in the patients with aseptic meningitis and only 2 to 3 days in those without "meningeal signs." Except for the shorter duration and the absence of neck or back stiffness, the patients in the minor illness group (45% of the illnesses in a surveyed population during this epidemic) presented essentially the same clinical manifestations as the aseptic meningitis group. Except for "mild to moderate lymphoid hyperplasia of the posterior pharynx" there were no positive findings on physical examination. The peripheral white blood counts were within normal limits. The number of cells in the cerebrospinal fluid of the patients with aseptic meningitis varied from 16 to 900 (average 200), of which approximately 80 to 97 per cent were lymphocytes, the sugar was normal, and the protein was only occasionally slightly elevated, the highest being 67 mg. per 100 ml.

Only about 50 per cent of the cases occurred in children under 14 years of age, and multiple familial cases were not uncommon. The incubation period could not be determined. While more than 50 per cent of the secondary cases in families occurred within 5 days, the longest interval was 21 days. No clinical data are available on the Swedish outbreak, except for the occurrence of an exanthem.

ECHO 6. This type of ECHO virus has been rarely encountered except in association with clinically recognized disease. At the Yale laboratory it was found only once among 212 enteric viruses recovered from healthy children (Melnick, 1955) and in the Cincinnati laboratory only once among more than 380 enteric cytopathogenic viruses recovered from healthy children in Cincinnati and Mexico.

The role of ECHO 6 virus in the aseptic meningitis syndrome is based on reports of its occurrence in 5 outbreaks between 1952 and 1955 in the U. S. and Sweden. In 1952 in Washington, D. C. (Habel et al., 1957)

ular study no ECHO 6 viruses were recovered from a control group of 296 ward and dispensary patients and 2 strains were recovered from 127 healthy children in the community. In 1954 in Sweden (von Zeipel and Svedmyr, 1957) 34 strains of ECHO 6 were found among 51 enteroviruses (4 polioviruses, 2 Coxsackie B 4, 2 Coxsackie A 7, 37 ECHO, 6 unidentified) recovered from 130 patients with a diagnosis of nonparalytic poliomyelitis or aseptic meningitis. During the same period no ECHO or Coxsackie viruses were found among 15 enteroviruses (all poliovirus) recovered from 33 patients with a diagnosis of paralytic poliomyelitis, and only 4 ECHO 6 viruses were found among 5 enteroviruses (1 Coxsackie B 4, 4 ECHO 6) and 1 adenovirus recovered from 128 individuals in a control group of healthy children or patients with other diagnoses. In 1954 in Boston (Kibrick et al., 1957) 40 strains of ECHO 6 were found among 65 enteroviruses (16 polioviruses, 5 Coxsackie, 43 ECHO, 1 unidentified) and 4 adenoviruses recovered from patients with a clinical diagnosis of poliomyelitis. In 1955, during the severe and extensive type 1 poliovirus epidemic in Boston ECHO 6 was again recovered from 10 patients with a diagnosis of nonparalytic poliomyelitis (Kibrick et al., 1957). In 1955 ECHO 6 was also prevalent in Connecticut (Davis and Melnick, 1956) where 21 strains were found among 69 enteroviruses (28 polioviruses, 16 Coxsackie, 25 ECHO) recovered from 137 patients with a

diagnosis of nonparalytic poliomyelitis, and only one strain among 45 enteroviruses (42 polioviruses, 1 ECHO 6, 1 Coxsackie A 9 and 1 Coxsackie B 4) recovered from 58 patients with a diagnosis of paralytic poliomyelitis. In western New York State in 1955, ECHO 6 was the predominating virus in an epidemic of "nonparalytic poliomyelitis," constituting more than 90 per cent of 172 cytopathogenic agents recovered in monkey kidney tissue cultures from patients and healthy household contacts (Karzon et al, 1956; Karzon, 1957). Ten strains of ECHO 6 virus were also isolated in 1955 from sporadic cases of aseptic meningitis in Washington, D C, California, Florida and Massachusetts (*Meyer et al., 1957*).

The clinical picture to be described here is based only on the manifestations observed in virologically proved cases (Davis and Melnick, 1956, Karzon et al, 1956; Kibrick et al, 1957, Meyer et al, 1957) The incubation period has been estimated at 3 to 5 days, based on the temporal sequence of cases within households (Kibrick et al, 1957). The disease affects children and adults The onset is characteristically abrupt with fever, severe headache and vomiting Sore throat was encountered in only 25 to 40 per cent of the patients Poorly defined abdominal pain was an occasional complaint, and diarrhea or constipation were absent or uncommon Stiffness of the neck and less often of the back appeared early in the majority of the patients and after an interval of several days of apparent well being in about 25 per cent of the patients, who exhibited a biphasic febrile and clinical course A convulsive onset was observed in a few patients, and generalized muscle pains were encountered with varying frequency in different outbreaks Fever was low-grade (100° to 102 6° F) and lasted 3 to 6 days Physical examination revealed mild or moderate stiffness of the neck or the back, or both, and occasional hamstring spasm Some depression of the superficial or deep tendon reflexes was occasionally found during the acute phase In the Holland, New York, outbreak where only ECHO 6 virus was recovered, some of the patients exhibited muscle weakness rated fair to poor by physiotherapists, at the time of discharge after several days of normal temperature, but no weakness, atrophy or spasm were found upon re-examination several months later (Karzon et al, 1956). In the 1954 Boston outbreak an orthopedic evaluation of 37 patients from whom only ECHO 6 virus was recovered designated the disease as paralytic in 22. Of the 22 cases classified as paralytic, 10 were placed in Grade I (score 0), i e., minimal weakness, 11 in Grade II (score 1 to 19) and 1 in Grade III (score 20 to 89) In most instances the mild to moderate degree of dysfunction disappeared with the passage of time. The muscles most frequently involved were the gluteus medius, the hip adductors and occasionally the gastrocnemius. Although it is possible that in a few instances there may have been concomitant infection with poliovirus, as was reported by others in isolated cases (Davis and Melnick, 1956; von Zeipel and Svedmyr, 1957), there were at least 3 patients with moderate muscle involvement in the Boston group, in whom this could be excluded because no neutralizing antibodies for any of the 3 types of poliomyelitis could be demonstrated (Kibrick et al, 1957).

There were no other significant clinical manifestations, adenopathy and splenomegaly were found in only 3 and 2 patients, respectively, in the Boston group None of the investigators noted the presence of rash, but during the summer of 1957 in Cincinnati a maculopapular eruption affecting the whole body, including the palms and the soles, was observed in one child, and a red, raised 2 to 3 mm. buccal lesion without exanthem, in its sibling during the course of a febrile illness associated with infection by ECHO 6 virus (Sabin et al, 1958b) In Sweden in 1954 serologic evidence of infection with ECHO 6 was observed in a patient with a diagnosis of erythema exudativum multiforme (von Zeipel and Svedmyr, 1957) *Febrile illnesses*, exhibiting all manifestations except those of meningeal irritation, as well as a few clinically inapparent infections, have been observed in household contacts of patients (Karzon et al, 1956; Kibrick et al, 1957)

The peripheral leukocyte counts were within normal limits or slightly elevated with neutrophils usually predominating. The leukocyte counts in the cerebrospinal fluid varied from 14 to 855 (the majority under 500) with polymorphonuclear cells predominating during the first few days after onset

and lymphocytes later on The concentrations of sugar and protein in the cerebrospinal fluid were either within normal limits or slightly elevated.

ECHO 9. The first strains of this type, originally designated as HE (human enteric) type 3, were recovered from healthy children during epidemiologically quiescent periods in Cincinnati and Mexico and represented only a small proportion of the large number of enteroviruses that were isolated (Ramos-Alvarez and Sabin, 1954, Ramos-Alvarez and Sabin, 1956) At the end of 1955 when this type of virus was renamed ECHO 9 there was only a single strain from a case of aseptic meningitis in West Virginia, and the importance of this virus as a cause of large epidemics of aseptic meningitis and febrile illnesses with rash did not become apparent until hundreds of strains recovered from patients in Belgium, Holland, Britain, Germany and Sweden in 1956 were almost simultaneously reported as being serologically similar to ECHO 9 (Nihoul and Quersin-Thiry, 1957, Johnsson, 1957, Sauthoff and Mittelstrass, 1957, McLean and Melnick, 1957; Boissard et al, 1957) ECHO 9 was subsequently also identified as the predominant virus in 1956 in epidemics in Switzerland (Baumann et al, 1957), Denmark (Godtfredsen and von Magnus, 1957) and Canada (Laforest et al., 1957) Viruses recovered from small outbreaks in Britain in 1954 and 1955 (Boissard et al, 1957) as well as those recovered only by tissue culture methods (Archetti et al, 1956) from the extensive outbreak in Italy in 1955 that was reported under the name of "Meningonevrassite Coxsackiosica Marchigiana" (Russi and Fua, 1956) have also been identified as related to ECHO 9 (Dalldorf, 1957b) In Germany other investigators reported the same disease either as being due to a new type of virus that was not pathogenic for mice (Hennessen, 1956) or as "Coxsackie Meningitis" (Brohl et al., 1957) because after several passages in tissue culture the viruses were pathogenic for mice as previously described. In 1957 extensive epidemics of ECHO 9 disease affecting tens of thousands of people were studied in the U.S.A (Sabin et al, 1958a).

The spectrum of the clinical manifestations of ECHO 9 disease, based only on virologically proved cases, definitely includes the aseptic meningitis syndrome and an undifferentiated febrile illness with or without exanthem and enanthem, and occasionally more extensive involvement of the central nervous system. The occurrence of exanthem which was a frequent finding in most of the European and American epidemics was not observed in the extensive Italian outbreak of 1955 (Archetti et al, 1956) or in the epidemic in a Swiss village in 1956 (Baumann et al, 1957), and it is possible that the incidence and the kind of clinical manifestations may vary with different strains of the virus.

The usual incubation period has been estimated at 5 to 8 days, but there is suggestive evidence that occasionally it may be as long as 15 to 20 days. The onset was invariably with headache, usually severe and frontal, generalized malaise, irritability and weakness. Fever (101° to 103° F.) appeared usually simultaneously with these symptoms or was delayed for 1 or 2 days, although patients who are not conscious of having fever may exhibit elevated temperatures Moderate chills were observed in 15 per cent of patients. Other symptoms include sore throat (about 50%), nausea and vomiting and occasionally generalized abdominal pain (more frequent in young children). Pain in the neck or the back or both occurs more often in older children and young adults and most frequently in those who develop objective signs of nuchal and spinal rigidity. Diarrhea, coryza and cough were not encountered in any of the patients A biphasic course, in which about 2 days of fever and illness are followed by a symptom-free and afebrile period of about 2 days and then by a return of fever and more severe symptoms was seen in about 25 per cent of the cases. A few patients exhibited a triphasic course. The temperature is usually continuously elevated or intermittent for from 2 to 15 days, the median number being 6. The pulse was either normal or only slightly elevated.

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was encountered The spleen was not palpable in any of the patients studied in Milwaukee or Cincinnati (Sabin et al, 1958a), while 33 per cent exhibited moderate enlargement during the Italian epidemic (Archetti et al, 1956) The liver extended 1 to 2 cm below the costal margin in about 4 per cent of the Milwaukee patients.

In the extensive Milwaukee epidemic of 1957 the incidence of rash decreased progressively with age Considering only virologically proved hospitalized and nonhospitalized cases a rash was seen in almost all children under 3 years of age, in about 70 per cent under 4 years, in 44 per cent of the 5- to 15-year group, and in only 7 per cent of those over 15 years of age In a detailed study of 43 cases, the rash appeared early after onset or within a few days after onset during the febrile period of the illness. It appeared generally first on the face or the face and the neck and within the ensuing 6 to 12 hours frequently spread to other parts of the body In 56 per cent of the cases the rash was present on the face, the neck, the trunk and the extremities, in 25 per cent only on the face, in 11 per cent on the face and the neck, and in 8 per cent on the face, the neck and the chest The rash is macular or maculopapular, faint pink to deep pink in color, and blanches on pressure The lesions are nearly always discrete, 1 to 3 mm in diameter, and only occasionally morbilliform, i e, confluent, with lesions up to 1 5 cm in diameter, usually on the face and the chest The rash persists for a day or less in some patients and for as long as 9 days in others, the median being 4 to 5 days The rash becomes pinkish-tan in color with the passage of time and does not desquamate A diffuse petechial rash, resembling that in meningococcemia, was observed in 3 patients during the Canadian outbreak (Laforest et al, 1957), although it was not stated that infection with ECHO 9 virus was specifically established in these 3 patients A petechial rash was also seen in one virologically proved case in Milwaukee.

An enanthem, with unilateral lesions occasionally resembling Koplik spots, was frequently present at the time of the exanthem, although in one large family infected with ECHO 9, only enanthem was found in 4 persons Conjunctivitis was present in 14 per cent of 76 virologically proved hospitalized patients

Patients were hospitalized, as a rule, because of nuchal or spinal rigidity or of marked pain in the neck or the back or both Actually, on physical examination only about 50 per cent of children under 10 years of age and about 65 per cent of those aged 10 to 40 years exhibited nuchal rigidity in the presence of a distinct pleocytosis of 10 cells or more Virus has been recovered from the cerebrospinal fluid of several patients who had only 5 cells per cu. mm and from that of patients without obvious nuchal rigidity with as many as 800 cells per cu mm (Sabin et al, 1958a). Spasm of the trapezius and other posterior cervical muscles when the chin touches the chest (Sabin's sign) may be detected in patients with pleocytosis in the absence of clear-cut nuchal or spinal rigidity, and it is clear that involvement of the central nervous system is much more frequent than suggested by the usual physical and laboratory criteria of meningeal irritation An epidemiologic and virologic survey in Milwaukee indicated that not less than 175 and probably as many as 300 ECHO 9 illnesses were at home for every ECHO 9 patient who was hospitalized because of suspected involvement of the central nervous system Since neck pain was encountered in 23 per cent of the patients under 10 and in 70 per cent of those over 10 who remained at home, it is probable that involvement of the central nervous system with few if any of the usual objective signs of meningeal irritation may be very common in ECHO 9 disease

A positive Kernig sign was noted in 9, a positive Brudzinski sign in 6, and a positive Babinski sign in only 3 of 76 hospitalized virologically proved cases These signs were noted only in children and were only transiently present Signs indicative of more extensive involvement of the central nervous system were observed in 5 virologically proved cases in the Milwaukee epidemic (Sabin et al, 1958a) Choreiform, jerking and undirected random movements of all extremities, right facial weakness and general facial grimacing were transiently present in a 1-month-old baby Coma and choreiform movements were noted in a 10-year-old girl. Loss of balance associated with an ataxic gait and tend-

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riant of ECHO 10 virus Human beings were shown to have an increasing incidence of antibody for this virus with increasing age, and the tissue culture passaged virus reproduced typical coryza in chimpanzees (Sabin, 1957a)

ECHO 16 Viruses recovered in 1951 in Massachusetts independently from 8 patients with a diagnosis of nonparalytic poliomyelitis (Kibrick et al, 1957) and from 7 of 18 persons with a febrile illness and rash (Neva et al, 1954) were subsequently classified as ECHO 16 (Committee on Enteroviruses, 1957). Another small outbreak of 24 cases of so-called "Boston Exanthem" disease, caused by the same virus, was identified in a Pittsburgh suburb in 1954 (Neva, 1956) The aseptic meningitis syndrome, as observed in virologically proved cases in Boston in 1951, was not associated with rash and was characterized by relatively abrupt onset, low-grade fever (100° to 101 8° F), severe, usually frontal headache, sore or red throat, vomiting, and moderate nuchal and spinal rigidity The CSF pleocytosis was usually less than 50 predominantly mononuclear cells and not more than 100 per cu mm , and the protein and the sugar were normal Recovery was rapid and without sequelae except in one 17-year-old boy who developed persistent spasm of the muscles of the back with only gradual improvement over the period of a year (Kibrick et al , 1957)

The striking feature about the febrile exanthem is that the rash usually did not appear for several hours to 2 days after the temperature returned to normal and the other signs disappeared The incubation period, based on histories of limited contact, has been established at 4 to 5 days The onset is more or less abrupt with mild sore throat, "stomachache," headache and occasionally reddened eyes Low-grade fever (100° to 103° F) is usually present and lasts only 1 or 2 days This is followed by a pink or salmon-colored, usually discrete macular or maculopapular eruption The lesions vary in size from a few mm to 1 cm and are usually distributed mostly on the face, the chest and the back; occasionally, the rash is more extensive, involving also the buttocks and the extremities, including the soles of the feet There is no pruritis The rash lasts only 2 to 4 days An enanthem, consisting of single or multiple, raised reddened areas or of tiny, yellowish-white, raised lesions, or both, on the soft palate, the fauces, or the uvula, was seen in nearly 50 per cent of the Pittsburgh patients, while herpanginalike lesions observed in a few of the Boston patients appear to have been associated with concomitant Coxsackie A infections Adenopathy was absent or not pronounced. Both children and adults were affected, and the disease tended to be more severe and rash less prominent in the adults. Although multiple cases of the exanthem disease occurred in a family, there were also instances of the febrile illness without rash, and, especially in infants, rash with very slight or negligible preceding elevation of temperature Both outbreaks occurred during the summertime Recovery was rapid, and none of the patients required hospitalization

ECHO 18. This virus was first encountered in an 8-month-old baby with a diarrheal disease during the summer of 1955 in Cincinnati (reported as the D 3 strain—Ramos-Alvarez, 1957). It was the only strain among 24 cytopathogenic agents recovered from 56 children with diarrhea in 1955, and it was not found at all among 41 cytopathogenic agents recovered from 97 children with diarrhea during the summer of 1956 in Cincinnati (Ramos-Alvarez and Sabin, 1958), but it proved to be the sole cause of an outbreak of diarrhea in a nursery for premature infants and in a ward for older full-term infants in the same hospital during the summer of 1956 in New York (Eichenwald et al , 1958). Twelve of the 21 premature infants, aged 6 to 46 days, and 5 babies, aged 1 week to 2 months, were affected. There was neither fever nor hypothermia The diarrhea, consisting of 5 to 6 fairly large, watery, greenish stools per day, persisted for 1 to 5 days with a mean of 3 days Neither mucus nor pus cells were present, but in 2 infants small flecks of bright blood were noted on a single occasion Physical examination of the 12 prematures was unremarkable except for moderate abdominal distention in 2 and a listless and lethargic appearance in 6 others The signs and symptoms were similar but somewhat more severe in the 5 full-term babies The same virus was recovered from 15 of the 17 sick children but from none of the children without illness on the same wards

ency to fall to the right appeared 2 days after onset and persisted for 10 days in a 22-month-old baby A clinical diagnosis of mild bulbar poliomyelitis was recorded on a 9-year-old boy who had received 3 doses of Salk poliomyelitis vaccine and exhibited a transitory nasal voice with diminished gag and cough reflexes. A diagnosis of spinal paralytic poliomyelitis was recorded on a 20-year-old girl who had received 2 doses of Salk vaccine and exhibited weakness and dizziness on standing, was unable to do sit-ups, and had enough tightness of the back muscles and weakness of the hips to require crutches for getting about for a period of about 2 months. In the last 2 patients no poliovirus could be detected in the stools tested in the presence of ECHO 9 antiserum.

Seventy virologically proved patients in Milwaukee were examined by experienced physiotherapists after defervescence prior to discharge from the hospital, varying degrees of spasm of the erector muscles of the neck were noted in 54 (severe in only 3) and some spasm of the thoracic or lumbar erector muscles in 48 (severe in 9) with frequent tightness of the hamstring muscles None exhibited flaccid paralysis, authough weakness of the abdominal muscles was noted in some of the patients who had severe spasm of the erector spinal muscles Where severe spasm was present there was a tendency for it to persist for many weeks

The occurrence of ECHO 9 virus in a few patients with mild transitory paralysis, in whom concomitant infection with poliovirus was excluded by serologic tests, was mentioned in a report from Switzerland (Baumann et al., 1957) The occurrence of more severe encephalitic and paralytic manifestations, including 3 deaths in old patients recorded during the 1955 epidemic in Italy (Archetti et al., 1956, Russi and Fua, 1956), was not confirmed by isolation of virus, and 68 per cent of 19 such patients tested were serologically negative However, during the 1956 epidemic in Holland, ECHO 9 virus was recovered in a concentration of about $10^{6.5}$ TCD_{50} per Gm. from the medulla of an 8-month-old infant who died within 24 hours after onset of an illness characterized by fever and coma (Verlinde, 1957).

CLINICAL LABORATORY FINDINGS. The cerebrospinal fluid (CSF) is as a rule under increased pressure and exhibits a pleocytosis which in certain epidemics has been reported as high as 6,000 (Archetti et al, 1956) or 8,448 cells per cu mm. (Baumann et al, 1957). In the majority of virologically proved Milwaukee patients (64/76) the CSF contained less than 1,000 cells; in 9 of 76 the number of cells was between 1,000 and 2,000, and in 3 it was over 2,000. Within 24 hours after onset the percentage of polymorphonuclear cells varied from 15 to 98, while subsequently 85 per cent or more of the cells were usually lymphocytes The pleocytosis has been noted to persist for at least 3 to 4 weeks without any increase in the amount of protein (Baumann et al, 1957). The CSF protein is usually within normal limits and only occasionally in the range of 75 to 120 mg per 100 ml. The CSF sugar is usually between 50 and 70 mg per 100 ml. In the peripheral blood the number of leukocytes is usually diminished or normal and only rarely increased The blood sedimentation rate has been found to be increased in the majority of patients during the acute phase (Archetti et al, 1956; Baumann et al, 1957; Sabin et al, 1958a). Nearly 50 per cent of the virologically proved Milwaukee patients exhibited a slight microscopic hematuria, and only 10 per cent had a low-grade proteinuria, both of which were transient During the Italian epidemic (Archetti et al, 1956) 16 per cent had a positive cephalin-cholesterol flocculation reaction, and 6 per cent exhibited urobilinuria, and a mild degree of azotemia was attributed to the increased CSF pressure rather than to renal insufficiency.

ECHO 10. The precise clinical picture produced by the many antigenic variants of this virus cannot as yet be defined. However, it has been shown to be associated with a family outbreak of "steatorrheic enteritis" in which 1 adult and 2 children (aged 4 and 6) exhibited varying degrees of fever, lower abdominal pain and frequent greasy (in one instance yellowish white) stools containing up to 50 per cent of fat on a dry weight basis A winter epidemic of typical coryza among chimpanzees, housed for several months in a Cincinnati laboratory, has been found to be etiologically associated with an antigenic va-

ated with a febrile illness characterized by rhinorrhea, sneezing, coughing, "discharge" from the eyes, vomiting and loose, malodorous stools (Rosen et al, 1958) Prototype strains of newly established ECHO types 21 and 22-24 were isolated from patients with aseptic meningitis and diarrhea, respectively

PATHOLOGIC PICTURE

The pathologic picture produced by ECHO viruses in human beings is still unknown While the report that ECHO type 2 was the only demonstrable virus in the CNS of a fatal case described as clinically and histologically typical of bulborespiratory poliomyelitis (Steigman et al, 1953, Steigman, 1957) must at present be interpreted with caution, nevertheless it suggests the need for further studies along this line, especially in the light of Verlinde's (1957) demonstration of a very high concentration of ECHO 9 virus in the medulla of another fatal case

Various types of lesions have been observed in the CNS of monkeys inoculated intraspinally or intracerebrally with certain types of ECHO virus In some instances (ECHO 7 and 8) these consisted mostly of focal interstitial infiltration in the gray matter of the medulla and the midbrain (Ramos-Alvarez and Sabin, 1954), in others (ECHO type 17) of localized areas of neuronal destruction and infiltration in the spinal cord comparable with those seen with attenuated strains of poliovirus, and in the case of ECHO 10 destructive lesions affecting especially the ependymal lining of the ventricles and the choroid plexus (Sabin, unpublished observations)

ECHO-9 virus has produced mild lesions in the spinal cord of monkeys inoculated directly into the lumbar area The lesions, including cellular infiltration and neuronophagia, were limited to the lumbar area of the spinal cord but were present outside the area of traumatic injury produced by the injection (Benyesh and Melnick, unpublished observations) Virus was recovered from the spinal cords of a number of monkeys for at least a week after inoculation of ECHO-9 virus Even more significant are the lesions in the CNS of occasional monkeys inoculated intramuscularly with ECHO types 1, 2, 3, 4, 6, 10 and 13 (Wenner and Chin, 1957) The lesions were focal, present most often in the lumbar and the cervical segments of the spinal cord, and were characterized by neuronal degeneration, ranging from moderate damage to complete absence of neurons, glial proliferation and infiltration of the gray matter with mononuclear cells In the ECHO 10 group, hyperplasia of the choroidal epithelium with infiltration by mononuclear cells and round cell infiltration of subependymal tissues were especially pronounced

EXPERIMENTAL INFECTION, HOST RANGE

For inclusion in the ECHO group prototype strains are tested in newborn mice, rabbits and monkeys, and none has produced clinically manifest disease in the numbers of animals usually used in such tests Since different strains of the same type or specially selected variants of a single strain may exhibit different properties, as was discussed for ECHO 9 and 10 earlier in this chapter, the full potentialities of the various types of ECHO virus still remain to be explored Clinically inapparent infections are probably not infrequent in monkeys, as was suggested by the occurrence of CNS lesions

Only inapparent infections resulted when 2 chimpanzees were fed 10^9 TCD_{50} of ECHO 6 and $2\frac{1}{2}$ months later $10^{7.8}$ TCD_{50} of ECHO 4 (Itoh and Melnick, 1957) Virus was recovered from the throat and the stools for a period of 10 to 12 days, and in 1 chimpanzee the type 4 virus was found in the throat for 35 days No virus was found in the blood, and both neutralizing and complement-fixing antibodies appeared as a result of the infection After parenteral injection of $10^{7.8}$ TCD_{50} of ECHO 6 into 2 nonimmune and 1 spontaneously immune chimpanzee, a transient, low-grade viremia was detected 2 days after inoculation in one of the chimpanzees, but no virus was found in the throat or the stools After parenteral injection of $10^{6.2}$ TCD_{50} of ECHO 4 virus in 2 chimpanzees, viremia was found only 24 hours after inoculation in one, in the stools of only one on the 2nd and the 3rd days and in the throat of the other only on the 4th day Inapparent infections were also demonstrated in single chimpanzees following the feeding of ECHO 2 and 3 viruses

A typical "common cold" coryzal syndrome was produced by nasal installation of the tissue culture passaged "chimpanzee-rhinitis"

All of the sick children developed antibody for this virus, but no antibody was found in the others While there was thus no evidence of inapparent infection in the infants, 2 of the nurses in the premature nursery were found to be healthy carriers, and one of these nurses was also implicated in the outbreak on the infant ward

Although the illness was not severe in these infants, ECHO 18 virus was recovered from the stool of a 5-month-old baby who died in Milwaukee during the summer of 1957 after an illness of 6 days' duration characterized by fever, repeated vomiting of bloody or coffee-ground material, slight enlargement of the liver and coma of 4 days' duration There were only 4 cells in the CSF, and the blood leukocyte count was 27,200 with 61 per cent neutrophils There was no necropsy, and one cannot be certain that the ECHO 18 virus was the cause of death (Sabin et al., 1958a) However, it is remarkable that thus far ECHO 18 virus has been associated with illness only in infants under 1 year of age.

Role of ECHO Viruses in Summer Diarrhea of Infants and Children. Studies carried out in Cincinnati during the summers of 1955 and 1956 on diarrheal disease in 153 children under 4 years of age indicated that enteroviruses can be recovered from nearly 50 per cent when single rectal swabs are tested, and limited serologic tests showed that the incidence of infection with these viruses is considerably higher (Ramos-Alvarez, 1957, Ramos-Alvarez and Sabin, 1958) Of the 65 cytopathogenic strains, 63 per cent were ECHO viruses, a simultaneous study in 1956 on a control group of 100 children matched for age, residence, socioeconomic status, and time of sampling showed that the incidence of ECHO viruses was 6 times higher among the infants and the children with diarrheal disease than among those without diarrhea at the time of sampling The 41 ECHO viruses recovered in 1955 and 1956 were made up of 13 distinct types (II-1; VI-7, VII-11, VIII-3; X-1; XI-2; XII-3; XIV-5; XVIII-1; XIX-1, and 6 strains belonging to 3 new types). This finding would suggest that diarrheal disease may be a frequent manifestation of infection in early childhood with many different types of ECHO viruses. Since type 18 has turned up as the sole cause of an isolated outbreak among very young infants, it is not improbable that the other types may be found to play similar roles on other occasions Among 34 children with diarrheal disease from whom only viruses were recovered, 62 per cent exhibited fever and vomiting; 44 per cent had mucus, and 20 per cent blood in the stools, 29 per cent exhibited dehydration, and in 53 per cent the illness was severe enough to require hospitalization. Among 13 children from whom both viruses and pathogenic *E coli* were recovered, the clinical manifestations were about the same except that 92 per cent were markedly dehydrated and required hospitalization None of the children died Summer diarrheal disease of early childhood may be regarded as a syndrome of multiple etiology, much like the syndrome of aseptic meningitis, and when tests were carried out for pathogenic enteric bacteria as well as for enteroviruses a potential etiologic agent was found in at least 85 per cent of the cases (Ramos-Alvarez and Sabin, 1958).

Role of Other ECHO Viruses. ECHO types 1, 13, 15 and 17 have thus far not been associated with disease. ECHO types 2, 3 and 5 have been recovered sporadically from patients with aseptic meningitis—type 5 from the spinal fluid as well as from stools (Melnick, 1957). ECHO type 7 has been recovered from healthy children, with relatively higher frequency from infants and young children with summer diarrhea (Ramos-Alvarez and Sabin, 1958), and only infrequently from patients with the aseptic meningitis syndrome (Habel et al., 1957; von Zeipel and Svedmyr, 1957). The precise role of types 8, 11 and 12 still remains to be elucidated, although type 8 has been associated with a febrile illness exhibiting clinical manifestations referable to both the respiratory and the alimentary tracts (Rosen et al., 1958). ECHO type 14 has been recovered sporadically from patients with aseptic meningitis, in one case from the spinal fluid (Melnick, 1957; Meyer et al, 1957; Ormsbee and Bell, 1957), in one instance associated with rash (Sabin et al., 1958), and from infants with summer diarrhea (Ramos-Alvarez and Sabin, 1958) ECHO type 20, represented by 100 strains recovered from young children in an orphanage in Washington, D. C., has been associ-

was little or no loss in activity after storage at 4° C. for 18 to 28 days for types 7, 8 and 11, while types 9 and 10 gradually lost activity This variation was more marked at room temperature (22° to 25° C), and with concentrations in the range of 100 TCD_{50}, virus was not detected at the end of 24 hours with types 7, 9 and 10, but types 8 and 11 persisted for more than a week While 90 per cent of the prototype ECHO 9 virus was lost in 3 days when approximately 100 TCD_{50} were stored at 4° C , stool extracts containing $10^{2.7}$ to $10^{3.4}$ TCD_{50} of 3 epidemic strains of ECHO 9 virus exhibited no loss in titer on storage at 4° C for 7 days (Eggers and Sabin, unpublished studies) A 50 to 90 per cent drop in titer was observed at the end of 24 hours at 37° C with ECHO types 6 and 7 and poliovirus type 1, while 99 9 per cent of Coxsackie A 9 was inactivated under the same conditions (Itoh, unpublished studies)

To avoid the accumulation of large amounts of heat-inactivated virus, which might yield misleading results in neutralization tests or interference phenomena with poor yields of virus in the case of slow-growing viruses, it is advisable to harvest tissue culture fluids as soon as most or all of the cells exhibit a cytopathogenic effect This is particularly important with such slow-growing and temperature-sensitive viruses as ECHO 10, 16 and 19 All specimens for isolation of ECHO virus should be stored at −20° C or lower temperatures as soon as possible after they are obtained from the patient Tissue culture fluids of all ECHO viruses can be shipped without refrigeration, but temperature-sensitive types (e g , 10, 16, 19) may need to be submitted to several rapid passages before maximal and optimum titers are obtained

ECHO viruses are not inhibited by the common antibiotics, i e , penicillin, streptomycin, tetracycline or mycostatin

Tissue cultures derived from the kidneys of rhesus and cynomolgus monkeys provide the cells of choice for the isolation and the optimum propagation of the ECHO viruses The cells derived from certain African species (Table 21), especially the green monkeys, *Cercopithecus aethiops sabaeus* (Drouhet, 1955), are as good or better for the

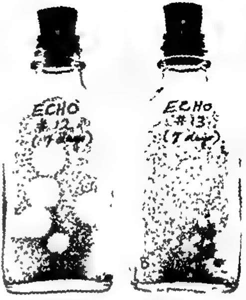

Fig 94 Plaques in monkey kidney monolayer cultures under agar, produced by ECHO 12 and ECHO 13 viruses, 7 days after seeding Plaques of ECHO 12 resemble those of wild strains of poliovirus (Hsiung, G D , and Melnick, J L , 1958, Orphan viruses of man and animals, Ann New York Acad Sc , *70*, 342-360)

types tested thus far, while those of the African red grass monkey (*Erythrocebus patas*) have been found satisfactory for ECHO types 7, 8 and 12, as well as for the Coxsackie B and polioviruses, and practically useless for ECHO types 1 to 6, 9, 11, 13 and 14, as well as for Coxsackie A 9 (Hsiung and Melnick, 1957b)

Cultures of human embryonic skin and muscle, postnatal uterus, or kidney tissue have been used for certain ECHO viruses (Kibrick et al , 1957) HeLa cells are unsuitable for the primary isolation of ECHO viruses, but after initial outgrowth and enrichment of the viral population it has been possible to adapt most of these viruses to growth in HeLa cells (Ramos-Alvarez and Sabin, 1956, Archetti et al , 1957, von Zeipel and Svedmyr, 1957, Ormsbee and Melnick, 1957) This selection of variants, possessing pathogenicity for HeLa cells, com-

TABLE 21 RELATIVE SUSCEPTIBILITY * OF KIDNEY CULTURES OF DIFFERENT MONKEY SPECIES TO REPRESENTATIVE ENTEROVIRUSES

MONKEY	POLIOVIRUS 2	COXSACKIE A9	COXSACKIE B1	ECHO 1	ECHO 7
Patas (*Erythrocebus patas*)	2.4	0.00000001	2.0	0.00000001	1.2
Spot-nose (*Cercopithecus nictitans buttikoferi*)	1.0	0.00000001		0.00000001	1.9
Green (*Cercopithecus aethiops sabaeus*)	1.9	1.7		2.4	2.3
Sooty mangabey (*Cercocebus fuliginosus*)	1.1	1.7		1.1	1.2
Olive baboon (*Papio doguera*)	1.3	1.3		1.1	1.3
Rhesus (*Macaca mulatta*)	1.0	1.0	1.0	1.0	1.0

* Relative susceptibility = $\frac{\text{Average plaque count in cultures of species tested}}{\text{Average plaque count in rhesus cultures}}$.

Data from Hsiung and Melnick (1957b)

virus (antigenic relative of ECHO 10) in 4 of 6 chimpanzees without preinoculation antibody. The virus was recovered from the nasal secretions and, in even greater concentration, from the stools of all 6 chimpanzees for as long as 10 to 14 days, and all developed neutralizing antibodies (Sabin and Schwarz, unpublished data)

ETIOLOGY

The extent to which the different types of virus now included in the ECHO group may vary in size is as yet unknown, because only 8 of the 24 established types have been measured Types 1, 2 and 3 (Melnick et al, 1953) only partly passed gradocol membranes of 38 mμ and were completely held back by membranes with an average pore diameter of 30 mμ Types 8, 9 and 11 passed 41 mμ membranes and were completely held back by the 27 mμ membranes, while the type 7 virus also passed the 27 to 29 mμ membranes, although only in minimal amounts (Ramos-Alvarez, unpublished data, Benyesh et al, 1958). On the basis of the Elford formula, which yields estimates that are smaller than those obtained by electron-microscopy, the above 7 viruses would have a size in the range of 10 to 15 mμ, i e, of the same order of magnitude (12 to 18 mμ) found by similar methods of measurement and estimation of size for the 3 types of poliovirus (Sabin et al., 1954). By similar methods, however, 3 different strains of ECHO 10 were found to have an estimated size of 60 to 90 mμ (Sabin, 1957). If a factor of 0 64 is used to convert limiting pore diameter, i e, that which just allows virus to pass, to particle size, the diameters obtained are in agreement with electronmicroscopic studies of those viruses obtained in crystalline arrays or of freeze-dried particles (Black, 1958). Application of this factor to the ECHO viruses studied, as well as to poliovirus, yielded particle diameters of 24 mμ (Benyesh et al, 1958)

Estimates of size based on the rate of inactivation by high energy electrons, alpha particles and deuterons yielded a value of 28 to 30 mμ for the infective unit and approximately 10 mμ for the complement-fixing antigenic unit for ECHO types 1 and 7, as well as for the 3 types of poliovirus (Benyesh et al, 1958)

Most ECHO viruses are relatively stable, so infected tissue culture fluids can be maintained at −20° C. for long periods of time without significant changes in titer. An exception to this rule is type 19, which is best preserved at −70° C The stability at 4° C and higher temperatures varies for different types and also for different strains of the same type. Tests on the prototype strains of types 7 to 11 (Ramos-Alvarez, unpublished studies) showed that at high concentrations of virus (10^5 to 10^7 TCD_{50} per ml.) there

6', certain strains of 8, 9, 11, 13, 14, 15, 16, 17, 18, 19) are irregular in shape and often small with diffuse boundaries (Figs 94 and 95) However, it has been found that different strains of the same type may produce different kinds of plaques, thus while the prototype, "Hill" strain of ECHO 9, produced only minute plaques (about 1 mm at 5 days), the "Vespa" strain derived from the 1955 epidemic in Italy yielded large plaques measuring about 11 mm at 5 days (Archetti, quoted by Wenner, 1957) In natural mixed infections small numbers of poliovirus particles may be isolated easily and quickly by passing progeny from the larger circular plaques (Fig 96)

FIG 96 Rhesus (*left*) and patas (*right*) cultures, 7 days after seeding with a stool specimen (D-11) from a patient experiencing a mixed viral infection On the rhesus culture, a single large poliovirus plaque (P) and 5 small ECHO plaques are present On the patas culture, only poliovirus plaques appear, because the cells are not susceptible to most ECHO viruses Progeny from the plaques were passed and typed in neutralization tests The large plaques yielded type 1 poliovirus, and the small plaques ECHO 17 virus (Hsiung, G D, and Melnick, J L, 1958, Orphan viruses of man and animals, Ann New York Acad Sc, *70*, 342-360)

Twenty different antigenic types (Table 22) have already been segregated from the huge mass of ECHO viruses, and many additional strains are in the process of classification (Committee on Enteroviruses, 1957) The different types were separated on the basis of cross-neutralization tests but similar results are also obtained by complement-fixation tests Cross-neutralization tests are necessary not only to guard against mixtures, which are also avoided by deriving the prototype strains from single clones (progeny obtained by plaque passage or from serial terminal dilutions), but also because of the not infrequent occurrence of so-called "prime" strains Such strains are neutralized to very low titer or not at all by the existing prototype antiserum, while an antiserum against the "prime" strain neutralizes the prototype virus to about the same titer as itself (Table 23) Naturally occurring strains with "prime" or other variations in antigenic relationship to the prototype strains are now known for types 5, 6, 7, 9, 10, 11 and 13 Varying degrees of antigenic interrelationship both by neutralization and CF tests have been found only for types 1, 8, 12 and 13 There is no common CF antigen for the ECHO group The possibility that some of the ECHO viruses may share a common antigen with the polioviruses requires further study with human sera, since it has been reported that persons without neutralizing antibodies for any of the 3 types of poliovirus developed CF antibodies for heat-inactivated poliovirus antigens after infection with ECHO 6 (von Zeipel and Svedmyr, 1957), or during a summer period when the antigenic stimulus was unknown (Melnick, 1955b)

Neutralizing antibodies are commonly determined by measuring the dilution of serum capable of preventing or markedly delaying the cytopathic effect of approximately 100 TCD_{50} of virus in tube cultures (Committee on the Enteroviruses, 1957) The metabolic inhibition test in panel cups under oil (Melnick and Opton, 1956) has also been used with certain strains The plaque reduction method has been especially useful with ECHO 4 virus, for which antibody is demonstrable

parable with the selection of mouse pathogenic variants in the case of ECHO 9 and 10, has not been achieved with ECHO 4 or all strains of the same type of virus, e.g., ECHO 9 (von Zeipel and Svedmyr, 1957; Archetti et al, 1957; Archetti, quoted by Wenner, 1957) Although fibroblastic cultures from human embryonic lung have been found suitable for primary isolation of many ECHO viruses (von Zeipel and Svedmyr, 1957), they were not suitable for certain epidemic ECHO 9 strains (Quersin-Thiry et al., 1957) Human amnion cells yielded satisfactory results with some ECHO 9 strains (McLean and Melnick, 1957, Quersin-Thiry et al., 1957) but completely failed to detect other ECHO 9 strains (Faulkner et al, 1957) and the ECHO 18 strains that were present in the stools of infants with diarrhea (Eichenwald et al., 1958), they were also only partly affected by ECHO 16 virus (Neva and Zuffante, 1957) The ECHO 16 virus has little or no effect on human kidney cells, and rhesus testicular cells are more sensitive than rhesus kidney cells

The ECHO 9 strains encountered in the 1956 epidemic in Belgium were reported (Quersin-Thiry et al., 1957) as being without effect on the following cell lines. HeLa, two strains of human kidney epithelium (adult and fetal), human lung fibroblasts, strain KB, conjunctiva, human intestine, human liver, mouse mammary carcinoma cells, and normal mouse kidney epithelium Pig kidney tissue cultures, which were found to be susceptible to all the Coxsackie B and adenoviruses, were not susceptible to ECHO types 1 to 9, 11, and 14 to 19 inclusive as well as to the Coxsackie A and polioviruses, ECHO 10 produced "some degree" of cytopathic effect (Guerin and Guerin, 1957)

ECHO types 2, 5, 10, and certain strains of types 3 and 6, have failed to produce plaques in rhesus or cynomolgus kidney tissue cultures under agar Types 7, 12, and certain strains of 8 produce large circular plaques with clear centers and sharp boundaries, similar to those of wild strains of polioviruses, on both rhesus and patas cells (Hsiung and Melnick, 1957a) The plaques of other ECHO viruses (1, 3, 4,

FIG. 95 Plaques of most ECHO viruses develop slowly. Those of ECHO 3, 4, 6, and 9 shown were photographed 11 days after seeding (Hsiung, G D, and Melnick, J. L, 1958, Orphan viruses of man and animals, Ann. New York Acad Sc., 70, 342-360)

ECHO 3, 6, 7, 10, 11 and 12 (as well as Coxsackie B 3) but not in those of ECHO 1, 2, 5, 8, 9, 13, 14 or in those of poliovirus types 1, 2, 3 or Coxsackie A 9 (Goldfield et al, 1957) ECHO 4 and the other Coxsackie B viruses have exhibited only slight hemagglutinating activity. The hemagglutinating property has been shown to be a function of the virus particle The hemagglutinin combines with the erythrocytes and can be eluted from them with exhaustion of the receptors for the same virus and to a certain degree also for the other viruses of the group (the receptor gradient being roughly in the order of ECHO 6, Coxsackie B 3, ECHO 3, 11, 7, 12 and 10), but not for influenza virus Treatment of Group O erythrocytes with receptor-destroying enzyme or influenza B virus (Lee) did not decrease their reactivity with the hemagglutinins of the enteroviruses Specific hemagglutination-inhibition antibody of high titer was demonstrated only in homotypic antisera, but the significance of the varying lower heterotypic titers cannot be evaluated until a method is found for removing the nonspecific inhibitors in various sera. These new findings on hemagglutinins seem to provide still another bridge between the ECHO and the Coxsackie viruses

DIAGNOSIS

Although there are no clues that might direct suspicion to ECHO viruses as a cause of disease in individual cases, the following epidemic situations during the summer and the autumn seasons may be regarded with suspicion:

1. A high incidence of the aseptic meningitis syndrome with few or no paralytic cases

2 Outbreaks of febrile illness associated with a high incidence of rash, especially in

TABLE 23 ‡ RELATIONSHIPS AMONG ECHO VIRUSES 6, 6′ AND 6″

VIRUS (100 TCD_{50})	Serum Tested: Type 6 Rh 7819	Type 6 Chimp 45	Type 6′ Rh 8922	Type 6″ Rh 8289
	A Serum Titer			
Type 6 (D'Amori)	320	64,000	600	60,000
Type 6′ (DiMeo)	10	150	300	2,400
Type 6″ (Burgess)	0 *	30	20	2,100
	B Neutralization Ratio †			
Type 6 (D'Amori)	1 0	1 0	2 0	28 6
Type 6′ (DiMeo)	0 03	0 002	1 0	1 1
Type 6″ (Burgess)	0 00	0 0005	0 07	1 0

* 0 = titer of less than 10

† Neutralization ratio = $\frac{\text{Titer against heterologous virus}}{\text{Titer against homologous virus}}$

(Homologous serum arbitrarily given a value of 1 0)

‡ Data from Melnick, 1957.

TABLE 22 LIST OF PROTOTYPE ECHO VIRUSES *

Type	Prototype Strain	Geographic Origin	Illness in Person Yielding Virus	Reference
1	Farouk	Egypt	None	Melnick, 1954, 1955
2	Cornelis	Connecticut	Aseptic Meningitis	Melnick, 1954, 1955
3	Morrisey	Connecticut	Aseptic Meningitis	Melnick, 1954, 1955
4	Pesascek	Connecticut	Aseptic Meningitis	Melnick, 1954, 1955
5	Noyce	Maine	Aseptic Meningitis	Melnick, 1954, 1955
6	D'Amori	Rhode Island	Aseptic Meningitis	Melnick, 1954, 1955
7	Wallace	Ohio	None	Ramos-Alvarez and Sabin, 1954, 1956
8	Bryson	Ohio	None	Ramos-Alvarez and Sabin, 1954, 1956
9	Hill	Ohio	None	Ramos-Alvarez and Sabin, 1954, 1956
10	Lang	Ohio	None	Ramos-Alvarez and Sabin, 1954, 1956
11	Gregory	Ohio	None	Ramos-Alvarez and Sabin, 1954, 1956
12	Travis 2-85	Philippine Islands	None	Hammon and Ludwig, 1955
13	Hamphill 2-188	Philippine Islands	None	Hammon and Ludwig, 1955
14	Tow	Rhode Island	Aseptic Meningitis	Melnick, 1957
15	Charleston 96-51	West Virginia	None	Ormsbee and Melnick, 1957
16	Harrington	Massachusetts	Aseptic Meningitis	Kibrick and Enders, 1957
17	CHHE-29	Mexico City	None	Ramos-Alvarez and Sabin, 1958
18	Metcalf	Cincinnati	Diarrhea	Ramos-Alvarez and Sabin, 1958
19	Burke	Cincinnati	Diarrhea	Ramos-Alvarez and Sabin, 1958
20	JV-1	Washington, D C	Fever	Rosen, 1958

* Types 21 to 24 have recently been added to this list

in only very low titers or not at all by the other methods (Itoh and Melnick, 1957) The culture fluids to be used for neutralization tests should be harvested early before heat-inactivated particles accumulate, because such inactivated virus may bind antibody and give low serum titers. The serum-virus mixtures are incubated for 1 hour at room temperature prior to addition to the culture tubes, and the amount of virus used in the test is checked by titrating the estimated 100 TCD_{50} after all the serum-virus mixtures have been added to the tubes.

Antigens for complement-fixation tests may be prepared from unheated monkey kidney tissue culture fluids (Melnick, 1955a, von Zeipel and Svedmyr, 1957, Hammon and Sather, unpublished observations). Potent antigens have been prepared in cultures maintained with C-E medium (0 01% cysteine, 0 25% glucose, Earle's salt solution) (Itoh and Melnick, 1957), but with the usual media these are often too anticomplementary to permit the use of an optimum amount of antigen Treatment with fluorocarbon has been recommended for removal of anticomplementary activity (Halonen, 1957), and more satisfactory and less anticomplementary antigens have been prepared from HeLa cells with adapted strains of virus (Archetti et al , 1957) Complement-fixation may be carried out by the micromethod on plates or by the conventional method in tubes.

Hemagglutinins of relatively high titer for human Group O erythrocytes have been reported in monkey kidney tissue cultures of

mon cells have been satisfactory for some but not all, and human kidney cells must be regarded as unsatisfactory for general use. After a cytopathogenic agent is recovered, a preliminary screening is carried out using a 10^{-4} dilution of culture fluid (titrating the virus simultaneously) and various pools of antisera against the 3 types of poliovirus, all available types of ECHO virus, and the cytopathogenic Coxsackie viruses (A 9 and B 1 to B 5). The final identification is made with individual antisera against 100 TCD_{50} of virus (Committee on Enteroviruses, 1957). In special cases in which it may be important to rule out or establish concurrent infection by 2 or more viruses, the original specimen is recultured in the presence of antiserum for one of the known or suspect viruses, and the acute and convalescent phase sera are tested for antibody against the suspected viruses. Tests on progeny from different types of plaques produced by the original specimens and inoculation of the original material into newborn mice are additional methods for detecting mixed infections.

TREATMENT

Treatment is symptomatic. Withdrawal of 15 to 20 ml of cerebrospinal fluid during the diagnostic lumbar puncture has been reported by several investigators (Archetti et al., 1956, Baumann et al., 1957) to relieve the very severe headache encountered in some patients with aseptic meningitis.

EPIDEMIOLOGY

The ECHO viruses are world-wide in distribution. They produce transitory infections of the human alimentary tract and are not comparable with the enteric bacteria which are constantly present in the intestinal tract. At least some of the viruses currently in the ECHO group (e.g., types 8, 10, 20) have been associated with clinical manifestations referable to the nasal mucosa. The incidence of ECHO viruses in the stools of healthy human beings, not in contact with acute febrile illnesses, has been shown to vary with age, season and socio-economic status. Tests on single rectal swabs from 1,566 healthy children during the summer of 1953 in Cincinnati indicated a carrier rate for ECHO viruses only of 5.2 per cent among those aged 1 to 4 years, 2.6 per cent in the 5 to 9 group, 0.2 per cent in the 10 to 14 group and none among 154 persons aged 15 to 17 (Ramos-Alvarez and Sabin, 1954). No ECHO viruses were recovered in repeated tests on stools of 100 men aged 20 to 30 years in Ohio (Sabin, 1957b). Using single rectal swabs for sampling, the incidence of nonpoliovirus cytopathogenic agents (more than 90% ECHO viruses) during late May and early June among 1,491 children aged 1 to 4 years in Mexico City was about 16 per cent (Ramos-Alvarez and Sabin, 1956). Repeated tests on the stools of 136 healthy, preschool children in Charleston, W. Va., over a 29-month period indicated that 90 per cent of the cytopathogenic enteroviruses were recovered during the summer and the autumn months and that the incidence was 3 to 6 times higher in the lower socio-economic district than in the middle-to-upper-middle-class districts; 52 per cent of these viruses belonged to the ECHO group (Honig et al., 1956). In areas further south such as Phoenix, Ariz. (Melnick, 1957) and Louisiana (Gelfand et al., 1957) the incidence of enteroviruses among healthy children was more evenly distributed throughout the year but still markedly higher during the months of May to October.

[illegible]

in an isolated community in Nova Scotia during the months of February and March (Faulkner et al., 1957). The size of the recorded outbreaks has varied from small groups to hundreds and many thousands, depending on the size of the community and the methods used for determining the incidence of infection with a specific ECHO virus. Epidemiologic surveys limited to hospi- [illegible]

studies are inadequate, since many enteroviruses are frequently being disseminated si- [illegible]

with only 27 reported cases of "nonparalytic

younger children, with or without a concomitant increase in the number of cases of aseptic meningitis

3 Outbreaks of diarrheal disease, especially in very young infants, from which few if any of the established enteropathogenic bacteria can be recovered.

Although thus far only the ECHO 4, 9 and 16 viruses have been associated with epidemics of febrile exanthem disease, sporadic febrile illnesses with rash have now been observed with infections caused by other ECHO (6, 14, and an as yet unclassified type) and Coxsackie (A 9 and B 4) viruses (Sabin et al, 1958b) and one cannot be certain that these or other members of the family of enteroviruses may not give rise to epidemics of exanthem disease at other times. The experience gained thus far may also provide the following clues regarding the viruses that may come under suspicion.

1 An outbreak in which the majority of the cases exhibit fever for not more than 2 days and the rash *follows* the fever by a day or two would suggest ECHO 16, while an outbreak characterized by fever of longer duration with rash appearing at the onset or during the febrile period would suggest ECHO 9 or some other agent

2 An outbreak of aseptic meningitis in which many patients exhibit a pleocytosis of 1,000 or more cells would suggest ECHO 9, even if rash were not a part of the clinical picture.

A specific virologic diagnosis is achieved most rapidly by isolation of virus from throat swabs, stools and cerebrospinal fluid, preferably all 3, when one is dealing with the aseptic meningitis syndrome Serologic tests, although useful for purposes of confirmation or under special conditions in individual cases, are impractical for rapid identification of the predominant viruses in an epidemic situation because the number of enteroviruses is too great. Properly obtained throat swabs (preferably 2 dry cotton swabs passed over the posterior pharyngeal wall and tonsillar fauces eluted in 2 to 4 ml. of tissue culture medium or Earle's solution containing 2,000 units of penicillin, 2 mg streptomycin and 250 units of mycostatin per ml) have proved to be an excellent source of virus in ECHO 9 (15 of 18 positive during first 3 days and 11 of 17 at 4 to 7 days—Sabin and Wigand, 1958; 29 of 41 during first 3 days—Archetti et al, 1956) and ECHO [illegible] infections (49 of 84 positive—Karzon, 1957a). Virus has been recovered from the throat in ECHO 4 and ECHO 16 infections, but the number of patients studied was too small to provide an indication of incidence. In the ECHO 18 outbreak, however, the virus could not be recovered from the throat of any of the infected infants or adult carriers The stools should be obtained for virus isolation whenever possible, but rectal swabs obtained in the same kind of medium as the throat swabs and requiring no preparation for inoculation in tissue cultures, can in many instances provide the most rapid means for identifying the predominating viruses The incidence of virus recovery from the stools of virologically proved cases (i e., by serology or isolation from other sources) has been approximately 85 per cent during the first week in patients with ECHO 6 and 9 infections and even higher in those with ECHO 16 and 18 infections The highest incidence of virus recovery from the cerebrospinal fluid has been in ECHO 9 infections (40 of 51 virologically proved cases—Sabin et al, 1958a, 47 of 90 tested during first week—Archetti et al., 1956); 7 strains of ECHO 6 have also been isolated from the CSF but the number tested was not indicated (Karzon, 1957). In addition, ECHO 5 and 14 viruses have each been recovered from the CSF (Melnick, 1957). The virus is only rarely present in the blood after onset of illness ECHO 9 virus was recovered from the serum of one contact tested 36 hours before onset of illness, from 1 of 6 patients tested within 24 hours after onset, and from none of 22 tested 2 to 6 days after onset (Sabin and Wigand, 1958) ECHO 16 virus has been recovered once from the blood clot of a patient with fever 2 days after onset of illness but not from [illegible] other afebrile patients tested 2 to 5 days after onset (Neva and Zuffante, 1957).

As was pointed out in a previous section, tissue cultures derived from the kidneys of rhesus or cynomolgus, and perhaps also green African, monkeys provide the cells of choice for isolation of most ECHO viruses Fibroblastic cultures from human embryonic lung have yielded good results, while human am-

in the stools of patients during the first week after onset was very small—less than 100 TCD_{50} per Gm in 65 per cent of 85 stools tested and usually only in the range of 10^2 to 10^3 TCD_{50} per Gm in the others The incidence of ECHO 9 virus in the stools of patients drops rapidly after the end of the second week—86 per cent (of 91) during the first week, 74 per cent (of 31) during the second week, 37 per cent (of 8) during the third and the fourth weeks, and only 5 per cent of 22, who were positive earlier, between the fifth and the seventh weeks—with only traces of virus being recovered in the stools obtained beyond the second week after onset (Sabin and Wigand, 1958)

CONTROL MEASURES

No specific control measures are available Until more [illegible] known about the effects of infection with various ECHO viruses on fetal development (Sabin et al, 1958a), it may be advisable for pregnant women, especially during the first trimester, to avoid all unnecessary intimate contact with children and adults exhibiting acute febrile illnesses, particularly those associated with rash, during the summer and the autumn months Avoidance of such contact may also be advisable for premature babies and very young infants During institutional outbreaks of viral, diarrheal disease among premature babies or infants, it may be helpful to identify possible carriers by testing the stools of the staff for cytopathogenic enteroviruses

REFERENCES

Archetti, I, Felici, A, Russi, F, and Fua, C, 1956, Researches on the etiologic agent of Marche meningoneuraxitis during the epidemic outbreak of the summer and autumn of 1955, Sc med ital *5*, 321-354

Archetti, I, Weston, J, and Wenner, [illegible] A, 1957, Adaptation of ECHO viruses in HeLa cells, their use in complement fixation, Proc Soc Exper Biol & Med *95*, 265-270

Baumann, T, Barben, M, Marti, R, Hassler, A, and Krech, U, 1957, Erkrankungen durch ECHO-Virus Typ 9 Eine epidemiologische, klinische and virologisch-serologische Studie, Schweiz med Wchnschr, *87*, 307-315

Benyesh, M, Pollard, E C, Opton, E M, Black, F L, Bellamy, W D, and Melnick, J L, 1958, Size and structure of ECHO, poliomyelitis, and measles viruses determined by ionizing radiation and ultrafiltration Virology, *5*, 256-274

Black, F L, 1958, Relationship between virus particle size and filterability through gradocol membranes Virology, *5*, 391-392

Boissard, G P B, Macrae, A D, Stokes, J L, and MacCallum, F O, 1957, Isolation of viruses related to ECHO virus type 9 from outbreaks of aseptic meningitis, Lancet *1*, 500

Brohl, I, Helmstaedt, K, Lennartz, H, and Maass, G, 1957, Über eine Epidemie von Coxsackie-Meningitis, Ztschr f arztliche Fortbildung *12*, 499-505

Chin, T. D Y, Beran, [illegible] W, and Wenner, H A, [illegible]

[illegible]

Committee on the ECHO Viruses, 1955, Enteric cytopathogenic human orphan (ECHO) viruses, Science *122*, 1187-1188

Committee on the Enteroviruses, 1957, The enteroviruses, Am J Pub Health *47*, 1556-1566

Dalldorf, G, 1957a, Brief review of Coxsackie viruses in 1956, Ann New York Acad Sc *67*, 209-211

———, 1957b, Discussion *in* Symposium on Cellular Biology, Nucleic Acids, and Viruses (Special Publication), vol 5, pp 390-392, New York Academy of Sciences

Davis, D C, and Melnick, J L, 1956, Association of ECHO virus type 6 with aseptic meningitis, Proc. Soc Exper Biol & Med *92*, 839 843

———, 1958, Poliomyelitis and aseptic meningitis A two year field and laboratory study in Connecticut J Lab [illegible] Clin Med *51*, 97-117

Droubet, V, 1955 Étude comparative de la sensibilite des cultures de tissu renal de *Cercopithecus aethiops sabaeus* et de *Macacus philippinensis* aux virus poliomyélitiques et aux autres virus entériques humains, Ann Inst Pasteur *89*, 666-671

Eggers, H J, and Sabin, A B, 1958, Factors determining mouse pathogenicity of ECHO [illegible] viruses, Fed Proc, *17*, 510

Eichenwald, H F, Ababio, A, Arky, A M, and Hartman, A P, 1958, Epidemic diarrhea in premature and older infants caused by ECHO virus type 18, J A M A, in press

Faulkner, E S, MacLeod, A J, and van Rooyen, C E, 1957, Virus meningitis—seven cases in one family, Canad M A J *77*, 439-444

Gelfand, H M, Fox, J P, and Leblanc, D R, 1957, The enteric viral flora of a population of normal children in Southern Louisiana, Am J Trop Med *6*, 521-531

Godtfredsen, A, and von Magnus, H, 1957, Isolation of ECHO virus type 9 from cerebrospinal fluids, Danish M Bull *4*, 233-236

Goldfield, M, [illegible]rihongse, S, and Fox, J P, 1957, Hemagglutinins associated with certain human enteric viruses, Proc Soc Exper Biol & Med *96*, 788 791

Guerin, L F, and Guerin, M M, 1957, Susceptibility

poliomyelitis," a survey of 500 persons in 143 families revealed that 16 per cent of the population had a compatible illness, and 55 per cent of the ill persons in the nonhospitalized group exhibited fever, headache and stiff neck or back (Lehan et al, 1957). Although only 21 of 57 individuals tested during this outbreak (including mostly patients with aseptic meningitis, some with minor illness or familial associates without illness) yielded cytopathogenic viruses, all 21 excreted ECHO 4 virus, and in 4 instances together with poliovirus (Chin et al, 1957). The incidence of ECHO 4 infection in families without illness was not determined, but the occurrence of inapparent infection was established in familial associates.

During the epidemic of ECHO 9 disease in Milwaukee in 1957, it was established that infection with this virus was almost entirely limited to families exhibiting compatible illnesses (Sabin et al, 1958a). Among 26 noncontact families with compatible illnesses during 1 week in August at the peak of the epidemic, tests on the stools of 104 ill and well persons yielded 66 cytopathogenic enteroviruses of which 58 were ECHO 9 During the same week stool specimens were obtained from 107 members of 25 families without illness, of similar socio-economic status and residing in the same neighborhoods as the families with illness, among the 10 cytopathogenic agents recovered from the 107 stools only 1 was ECHO 9 During the same week a survey was made of 2,447 households containing 11,403 persons (about 1 5% of the total population of 740,000 in Milwaukee) and 62 households (including the 26 studied virologically) had one or more persons with compatible illnesses A crude estimate suggested that not less than 175 and probably as many as 300 people were ill with ECHO 9 disease for every one that was hospitalized During the course of the Milwaukee epidemic, extending from the end of June to the end of September (confirmed by isolation of ECHO 9 at both extremes) only 149 residents of the city were hospitalized, but actually the epidemic may have affected approximately 45,000 persons, i e, an estimated attack rate of only 6 per cent compared with the estimated attack rate of about 16 per cent for the epidemic of ECHO 4 disease in Marshalltown, Iowa Among the families with ECHO 9 infection, the rate of inapparent or unrecognized infection (based only on recovery of virus in the absence of recent, current or subsequent illness) was 18 per cent, while the rate of compatible illnesses was even higher (Sabin et al, 1958a).

The age incidence of disease observed during epidemics of ECHO 4, 6, 9 and 16 viruses indicates that all groups are attacked, apparently in proportion to the incidence of pre-existing immunity An exception to this rule is ECHO 18, which thus far has affected only infants under 1 year of age. An enterovirus antibody survey in Connecticut indicated that antibodies against ECHO viruses were not as prevalent as those against the polioviruses (Davis and Melnick, 1958). Among those under 15 years of age, the per cent positive for the ECHO types studied were: II—19, V—6, VI—17, IX—9, while among the adults the corresponding figures were II—36, V—16, VI—23 and IX—19. A preponderance of males among patients with aseptic meningitis in a ratio of about 2 5:1 has been observed in ECHO 6 (Karzon, 1958) and about 2:1 in ECHO 9 infections (Archetti et al., 1956, Baumann et al, 1957, Sabin et al, 1958a) Multiple cases in families, particularly in those with many young children, are the rule rather than the exception While secondary cases in families occur for the most part during the first week, longer intervals up to 15 and 20 days have also been observed Observations of families into which different enteroviruses were introduced under natural conditions demonstrated that ECHO 6, Coxsackie A9, B3, and poliovirus spread through the susceptibles with equal ease (Davis and Melnick, 1958) In the course of this study, confirmation was obtained of the effect of Salk vaccine in increasing the poliomyelitis antibody prevalence and decreasing the incidence of poliomyelitis in the vaccinated group In contrast, the vaccine had no effect on the prevalence of aseptic meningitis due to Coxsackie or ECHO viruses

Dissemination by fecal contamination appears to be the most important mode of spread for the epidemic ECHO viruses studied thus far Among 8 patients who had varying amounts (10^1 to $10^{5.2}$ TCD_{50} per swab) of ECHO 9 virus in their throats during the acute phase of their illness, none had demonstrable virus in swabs from the nose, and the only patient to yield any virus ($10^{1.8}$ TCD_{50} per swab) in swabs from the anterior buccal mucosa, tongue and gums was the one who had $10^{5.2}$ TCD_{50} per swab in the throat (Sabin and Wigand, 1958). The stools may be most infectious before onset of illness, e g, the amount of ECHO 9 virus found

in the stools of patients during the first week after onset was very small—less than 100 TCD_{50} per Gm in 65 per cent of 85 stools tested and usually only in the range of 10^2 to 10^3 TCD_{50} per Gm in the others The incidence of ECHO 9 virus in the stools of patients drops rapidly after the end of the second week—86 per cent (of 91) during the first week, 74 per cent (of 31) during the second week, 37 per cent (of 8) during the third and the fourth weeks, and only 5 per cent of 22, who were positive earlier, between the fifth and the seventh weeks—with only traces of virus being recovered in the stools obtained beyond the second week after onset (Sabin and Wigand, 1958)

CONTROL MEASURES

No specific control measures are available Until more is known about the effects of infection with various ECHO viruses on fetal development (Sabin et al, 1958a), it may be advisable for pregnant women, especially during the first trimester, to avoid all unnecessary intimate contact with children and adults exhibiting acute febrile illnesses, particularly those associated with rash, during the summer and the autumn months Avoidance of such contact may also be advisable for premature babies and very young infants During institutional outbreaks of viral, diarrheal disease among premature babies or infants, it may be helpful to identify possible carriers by testing the stools of the staff for cytopathogenic enteroviruses

REFERENCES

Archetti, I, Felici, A, Russi, F, and Fua, C, 1956, Researches on the etiologic agent of Marche meningoneuraxitis during the epidemic outbreak of the summer and autumn of 1955, Sc med ital 5, 321-354

Archetti, I, Weston, J, and Wenner, H A, 1957, Adaptation of ECHO viruses in HeLa cells, their use in complement fixation, Proc Soc Exper Biol & Med 95, 265-270

Baumann, T, Barben, M, Marti, R, Hassler, A, and Krech, U, 1957, Erkrankungen durch ECHO-Virus Typ 9 Eine epidemiologische, klinische und virologisch-serologische Studie, Schweiz med Wchnschr, 87, 307-315

Benyesh, M, Pollard, E C, Opton, E M, Black, F L, Bellamy, W D, and Melnick, J L, 1958, Size and structure of ECHO, poliomyelitis, and measles viruses determined by ionizing radiation and ultrafiltration Virology, 5, 256-274

Black, F L, 1958, Relationship between virus particle size and filterability through gradocol membranes Virology, 5, 391-392

Boissard, G P B, Macrae, A D, Stokes, J L, and MacCallum, F O, 1957, Isolation of viruses related to ECHO virus type 9 from outbreaks of

... J Hyg 66, 76-84

Committee on the ECHO Viruses, 1955, Enteric cytopathogenic human orphan (ECHO) viruses, Science 122, 1187-1188

Committee on the Enteroviruses, 1957, The enteroviruses, Am J Pub Health 47, 1556-1566

Dalldorf, G, 1957a, Brief review of Coxsackie viruses in 1956, Ann New York Acad Sc 67, 209-211

———, 1957b, Discussion in Symposium on Cellular Biology, Nucleic Acids, and Viruses (Special Publication), vol 5, pp 390-392, New York Academy of Sciences

Davis D C and Melnick J L ...

—

Dr..., ..., 1955 Etude comparative de la sensi-

Fed Proc, 17, 510

Eichenwald, H F, Ababio, A, Arky, A M, and Hartman, A P, 1958, Epidemic diarrhea in premature and older infants caused by ECHO virus type 18, J.A.M.A, in press

Faulkner, E S, MacLeod, A J, and van Rooyen, C E, 1957, Virus meningitis—seven cases in one family, Canad M A J 77, 439-444

Gelfand, H M, Fox, J P, and Leblanc, D R, 1957, The enteric viral flora of a population of normal children in Southern Louisiana, Am J Trop Med 6, 521 531

Godtfredsen, A, and von Magnus, H, 1957, Isolation of ECHO virus type 9 from cerebrospinal fluids, Danish M Bull 4, 233-236

Goldfield, M, Srihongse, S, and Fox, J P, 1957, Hemagglutinins associated with certain human enteric viruses, Proc Soc Exper Biol & Med 96, 788-791

Guerin, L F, and Guerin, M M, 1957, Susceptibility of pig tissue cultures to certain viruses, Proc Soc Exper Biol & Med 96, 322-323

Habel, K, Silverberg, R J, and Shelokov, A, 1957, Isolation of enteric viruses from cases of aseptic meningitis, Ann New York Acad Sc 67, 223-229

Halonen, P, 1957, The homologous and heterologous complement fixation titers of ECHO antisera prepared in monkeys, in manuscript

Hennessen, W, 1956, Some features of a new type of virus meningitis, Deutsche med Wchnschr. *81*, 2088-2090

Honig, [redacted] I, Melnick, J L, Isacson, P, Parr, R, Myers, I L, and Walton, M, 1956, An endemiological study of enteric virus infections—poliomyelitis, Coxsackie, and orphan (ECHO) viruses isolated from normal children in two socio-economic groups, J Exper Med *103*, 247-262

Hsiung, G D, and Melnick, J L, 1957a, Morphologic characteristics of plaques produced on monkey kidney monolayer cultures by enteric viruses (poliomyelitis, Coxsackie, and ECHO groups), J Immunol *78*, 128-136

———, 1957b, Comparative susceptibility of kidney cells from different monkey species to enteric viruses (poliomyelitis, Coxsackie, and ECHO groups), J Immunol *78*, 137-146

Itoh, H, and Melnick, J L, 1957, The infection of chimpanzees with ECHO viruses, J Exper Med *106*, 677-688

Johnsson, T, 1957, A new clinical entity? Lancet *1*, 590

Karzon, D T, 1957a (Discussion) Viruses in search of disease, Ann New York Acad Sc *67*, 351-352

———, 1958, Outbreaks of aseptic meningitis caused by type 6 ECHO virus *in* Poliomyelitis: Papers and Discussions Presented at the Fourth International Poliomyelitis Conference, Philadelphia, Lippincott, pp 203-206

Karzon, D T, Barron, A L, Winkelstein, W, Jr, and Cohen, S, 1956, Isolation of ECHO virus type 6 during outbreak of seasonal aseptic meningitis, J A M A *162*, 1298-1303

Kibrick, S, Melendez, L, and Enders, J F, 1957, Clinical associations of enteric viruses with particular reference to agents exhibiting properties of the ECHO group, Ann New York Acad Sc *67*, 311-325

Laforest, R A, McNaughton, G A, Beale, A J, Clarke, M, Davis, N, Sultanian, I, and Rhodes, A J, 1957, Outbreak of aseptic meningitis (meningoencephalitis) with rubelliform rash Toronto, 1956, Canad M A J *77*, 1-4

Lehan, P H, Chick, E W, Doto, I L, Chin, T D Y, Heeren, R H, and Furculow, M L, 1957, An epidemic illness associated with a recently recognized enteric virus (ECHO virus type 4), I Epidemiologic and clinical features, Am J Hyg *66*, 63-75

Ludwig, [redacted] H, and Hammon, W McD, 1956, ECHO viruses isolated in a survey of normal persons, Luzon, Philippine Islands, Fed Proc *15*, 601

McLean, D M, and Melnick, J L, 1957, Association of a mouse pathogenic strain of ECHO virus type 9 with aseptic meningitis, Proc Soc Exper Biol & Med *94*, 656-660

Melnick, J L, 1954, Application of tissue culture methods to epidemiological studies of poliomyelitis, Am J Pub Health *44*, 571-580

———, 1955a, Tissue culture techniques and their application to original isolation, growth, and assay of poliomyelitis and orphan viruses, Ann New York Acad Sc *61*, 754-772

———, 1955b, Isolation of virus and the development of neutralizing and complement-fixing antibodies in poliomyelitic patients, Ann New York Acad Sc *61*, 1005-1010

———, 1957, ECHO viruses *in* Cellular Biology, Nucleic Acids and Viruses (Special Publication), vol 5, pp 365-381, New York Academy of Sciences

Melnick, J L, and Agren, K, 1952, Poliomyelitis and Coxsackie viruses isolated from normal infants in Egypt, Proc Soc Exper Biol & Med *81*, 621-624

Melnick, J L, and Opton, E M, 1956, Assay of poliomyelitis neutralizing antibody in disposable plastic panels, Bull World Health Organ *14*, 129-146

Melnick, J L, Riordan, J T, Curnen, E C, and Macrae, A D, 1953, Tissue culture pathogenic agents other than poliomyelitis virus isolated from cases of suspected poliomyelitis, Fed Proc *12*, 454

Meyer, H M, Jr, Rogers, N G, Miesse, M L, and Crawford, I P, 1957, Aseptic meningitis caused by orphan viruses and other agents, Ann New York Acad Sc *67*, 332-337

Neva, F A, 1956, A second outbreak of Boston exanthem disease in Pittsburgh during 1954, New England J Med *254*, 838-843

Neva, F A, Feemster, R F, and Gorbach, I J, 1954, Clinical and epidemiologic features of an unusual epidemic exanthem, J A M A *155*, 544-548

Neva, F A, and Zuffante, S M, 1957, Agents isolated from Boston exanthem disease during 1954 in Pittsburgh, J Lab & Clin Med *50*, 712-719

Nihoul, E, and Quersin-Thiry, L, 1957, A new clinical entity? Lancet *1*, 269-270

Ormsbee, R A, and Bell, E J, 1957, ECHO viruses from Idaho and Montana, Am J Pub Health *47*, 1405-1413

Ormsbee, R A, and Melnick, J L, 1957, Biologic and serologic characteristics of ECHO viruses from West Virginia, J Immunol *79*, 384-392

Quersin-Thiry, L, Nihoul, E, and Dekking, F, 1957, ECHO virus type 9 (new member of Coxsackie group type A?) as a cause of epidemic meningitis, Science *125*, 744-745

Ramos-Alvarez, M, 1957, Cytopathogenic enteric viruses associated with undifferentiated diarrheal syndromes in early childhood, Ann New York Acad Sc *67*, 326-331

Ramos-Alvarez, M, and Sabin, A B, 1954, Characteristics of poliomyelitis and other enteric viruses recovered in tissue culture from healthy American children, Proc Soc Exper Biol & Med *87*, 655-661

———, 1956, Intestinal viral flora of healthy children demonstrable by monkey kidney tissue culture, Am J Pub Health *46*, 295-299

———, 1958, Role of enteropathogenic viruses and bacteria in summer diarrheal diseases of infancy and early childhood, J A M A *167*, 147-156

Robbins, F C, Enders, J F, Weller, T H, and Florentino, G L, 1951, Studies on the cultivation

of poliomyelitis viruses in tissue culture V The direct isolation and serologic identification of virus strains in tissue culture from patients with nonparalytic and paralytic poliomyelitis Am J Hyg 54, 286-293

Rosen, L, Johnson, J H, Huebner, R J, and Bell, J A, 1958, Observations on a newly recognized ECHO virus and a description of an outbreak in a nursery In manuscript

Russi, F, and Fua, C, 1956, L'incidenza dell'accertamento virologico nelle varie forme cliniche della meningoneviassite coxsackiosa Marchigiana, Policlinico (sez prat) 63, 1-3

Sabin, A B, 1957a (Discussion) Viruses in search of disease, Ann New York Acad Sc 67, 250-252

———, 1957b (Discussion) Viruses in search of disease, Ann New York Acad Sc 67, 349-351

Sabin, A B, Hennessen, W A, and Warren, J, 1954, Ultrafiltration and electron microscopy of three types of poliomyelitis virus propagated in tissue culture, Proc Soc Exper Biol & Med 85, 359-363

Sabin, A B, Krumbiegel, E R, and Wigand, R, 1958a, ECHO 9 virus disease Virologically controlled clinical and epidemiologic observations during 1957 epidemic in Milwaukee To be published

Sabin, A B, and Wigand, R, 1958, Properties and behavior of ECHO 9 viruses recovered in Milwaukee and Cincinnati in 1957 To be published

Sabin, A B, Wigand, R, and Smith, H D, 1958b, Etiology of summer febrile illnesses with rash in Cincinnati in 1957 To be published

Sauthoff, R, and Mittelstrass, H K, 1957, Neutralisierende Antikörper in Menschlichen Seren gegen ECHO Virus Typ 9, Klin Wchnschr 35, 311-312

Steigman, A J, 1957 (Discussion) Viruses in search of disease, Ann New York Acad Sc 67, 249-250

Steigman, A J, Kokko, U P, and Silverberg, R J, 1953, Unusual properties of a virus isolated from the spinal cord of a child with fatal poliomyelitis, A M A Am J Dis Child 86, 500-510

Verlinde, J D, 1956, Discussion in Poliomyelitis Papers and Discussions Presented at the Fourth International Poliomyelitis Conference, Philadelphia, Lippincott

Wenner, H A, 1958, Benign aseptic meningitis in Poliomyelitis Papers and Discussions Presented at the Fourth International Poliomyelitis Conference, Philadelphia, Lippincott

Wenner, H A, and Chin, T D Y, 1957, Discussion in Symposium on Cellular Biology, Nucleic Acids, and Viruses (Special Publication), vol 5, pp 384-387, New York Academy of Sciences

von Zeipel, G, and Svedmyr, A, 1957, A study of the association of ECHO viruses to aseptic meningitis, Arch ges Virusforsch 7, 355-368

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27

Infectious Hepatitis and Serum Hepatitis

There are probably several viruses that cause hepatitis in man, and certain of them may be variants of one general group Two major members of this group, known as hepatitis virus A and virus B, are the causative agents of *infectious hepatitis* and *serum hepatitis*, respectively Synonyms for the former disease are *infective hepatitis* and *epidemic jaundice*, and for the latter, *homologous serum jaundice, syringe jaundice, postvaccinal hepatitis, transfusion jaundice*, etc. Presumably, *infectious hepatitis* is an example of the so-called "enteric group of infectious diseases" *Serum hepatitis*, on the other hand, has many features that do not belong in the enteric category and, epidemiologically speaking, are almost unique. Although there is scant knowledge concerning the exact relationship between these 2 viruses (A and B) or groups of viruses, some kind of distinction between them seems to be desirable The properties on which the differentiation is based are not well defined but can be discussed in terms of certain epidemiologic, immunologic and clinical aspects of the illnesses that virus A and virus B cause.

INFECTIOUS HEPATITIS

Introduction

Infectious hepatitis is a relatively common and widespread acute or subacute viral infection in which there is a diffuse involvement of the liver. It appears in both endemic and epidemic form Clinically, it is characterized in the early stage by fever, anorexia, nausea, vomiting and abdominal distress, with subsequent hepatomegaly and jaundice. In children the illness may be brief, but in adults it usually lasts 3 to 8 weeks It is thought to be spread by the oral-enteric route and, in general, to give rise to homologous immunity.

History

Infectious hepatitis has long been recognized in civilian and military medical history under a variety of different names. Its present-day prominence stems not only from its current frequency and occasional seriousness but also from the development of new concepts of pathogenesis and the significance attached to a new name. For, as long as *infectious hepatitis* went under such names as *epidemic jaundice* or *acute catarrhal jaundice* (both regarded as mild illnesses) or *acute yellow atrophy of the liver* (regarded as a rare although very serious disease), it occupied a place of little importance in medical thought This was its situation prior to 1940 *Now not only is it regarded as a common* virus disease with a name emphasizing the idea of contagion but also it carries the possibility of moderately serious implications in adults. Much of this new concept of its pathogenesis and severity arose when the idea gained credence that this type of jaundice was not due to obstruction of the common bile duct with a mucous plug but rather to a destructive lesion of the hepatic parenchyma. The degree to which this disease

proved to be a scourge to troops during World War II further magnified its importance

Prior to the 1880's, epidemic jaundice was often confused with Weil's disease. Originally differentiated from infectious hepatitis on clinical grounds in 1886, cases of leptospirosis have more recently been clearly diagnosed by immunologic tests Later pioneers in clarifying the identity of infectious hepatitis as a clinical entity include Quincke, 1903, and Cockayne, 1912 Blumer, 1923, was among the first in the United States to point out that infectious hepatitis probably represented the epidemic form of "catarrhal jaundice" Rich, 1930, again drew attention to the fact that at necropsy nearly all cases of "catarrhal jaundice" showed a diffuse hepatitis

Modern concepts of the viral etiology of hepatitis developed about 1939 when various observers (Findlay et al, 1939) in West Africa described cases of postvaccinal (yellow fever vaccine) hepatitis This is now commonly called serum hepatitis and, although its existence had been recognized since the 1880's and repeatedly described since then, Findlay et al (1939) were apparently the first to conclude that the causal agent was a virus In 1942, Voegt, in Germany, was the first to report the oral transmission of infectious hepatitis (virus A) from man to man by feeding duodenal contents from a patient suffering from this illness. Subsequently, others demonstrated the causative agent in the blood (Cameron, 1943) and the feces (MacCallum and Bradley, 1944, Havens et al, 1944, Neefe et al, 1946) and showed that it was capable of passing bacteria-tight filters and was transmissible serially in volunteers (Havens, 1945a). Nevertheless, this agent has not yet been "isolated" in the sense that it has been seen or cultured or definitely transmitted to laboratory animals, and its properties that are now known have been demonstrated entirely in volunteers

Clinical Picture

The incubation period ranges from 15 to 40 days, with an average of about 25 In childhood, the course of disease is shorter and milder than it is in adults (Horstmann et al., 1947), and in young infants it may be so mild

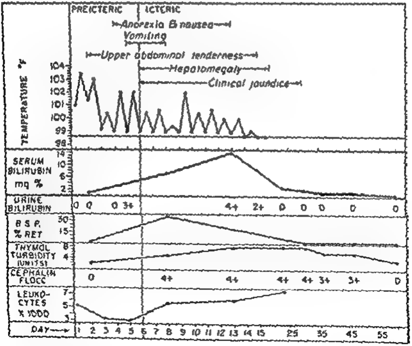

Fig. 97 The clinical course of infectious hepatitis in an experimentally infected volunteer

as to escape notice (Capps et al, 1950). In both children and adults, but particularly in *the former, jaundice may be absent or quite* fleeting Estimates of the ratio of nonicteric to icteric cases differ in various epidemics, but a conservative ratio in adults would be 1 1. The course of disease in adults is usually, and somewhat arbitrarily, separated into 2 phases: preicteric and icteric (see Fig. 97) Each *phase has characteristic symptoms, signs and* laboratory findings Actually, the great majority of patients with hepatitis are already in *the icteric phase by the time the diagnosis is* made, so that the clinical aspects of the second phase are more familiar to most physicians than are those of the first phase

The preicteric phase, which may range from 1 to 21 days, may start abruptly or insidiously with symptoms such as anorexia, nausea, moderate abdominal discomfort and diarrhea A large proportion of patients have fever at this time, often accompanied by chills or chilly sensations Posterior cervical lymphadenopathy ■ common (Barker et al, 1945), and splenomegaly may be present Although the liver is not usually enlarged in this stage, tenderness may often be elicited by palpation Leukopenia is a characteristic finding at this time, and toward the end of the preicteric phase many large atypical lymphocytes, similar to those found in patients with infectious mononucleosis, are often seen (Havens and Marck, 1946). Many patients have subjective improvement toward the end of the preicteric phase, lasting 1 or 2 days until jaundice appears

The icteric phase is ushered in with a return of gastro-intestinal symptoms It may last in adults from 1 to 10 weeks, averaging 6 weeks Some patients may have slight jaundice for several months Major symptoms and signs in the icteric phase are again anorexia, abdominal discomfort, usually related to pain in the right upper quadrant or epigastrium, nausea and often vomiting The liver becomes enlarged and more tender and usually is easily palpable. The spleen is palpable and tender in a fair percentage of patients These symptoms and signs may last only a short time or as long as a month, although usually improvement occurs within 2 weeks at the time when jaundice has reached its maximum intensity and has begun to wane. Considerable loss of weight is not uncommon, but ■ jaundice diminishes, a sense of well-being and appetite return Convalescence is generally uneventful and rapid. Fatalities are uncommon, and the mortality ranges from 1 to 3 per 1,000. As in a number of virus diseases, the older the patient the more severe the illness and the higher the mortality (Muller, 1947, *Sherman and Eichenwald, 1956*). *Death* may occur early in the disease, after 3 to 10 days of illness, or later, 3 to 8 weeks after onset *Sudden appearance of restlessness, mental con-*fusion, loss of emotional control, coma and hemorrhagic phenomena carries a grave prognosis True complications are rare, but occasionally patients develop pneumonia and, very rarely, agranulocytosis, lymphocytic meningitis or myelitis The frequency of relapse has been reported to range from 0 6 to as much as 18 per cent of the adult cases, although the high rates are most uncommon In a fair percentage of so-called "relapses," the only evidence is furnishd by laboratory tests that indicate a mild worsening of hepatic function. In another group of patients, a relapse manifests itself by a return of symptoms or physical signs of the icteric phase of disease, although it is usually less severe than the initial illness Complete recovery occurs in most patients both with or without relapses

A few patients have hepatic dysfunction prolonged beyond the usually expected time of recovery, with or without signs or symp-

bromsulphalein (Post et al, 1950) A large proportion of this group are asymptomatic, and it is questionable to what degree the slightly abnormal tests of hepatic function should be regarded as actual manifestations of illness In most of these cases, the degree of functional impairment of the liver and the clinical condition are apparently compatible with full activity and eventual complete recovery (Klatskin and Rappaport, 1947; Kunkel et al, 1947) Another smaller group of patients have more prolonged anorexia, lassitude and epigastric discomfort, with jaundice, enlargement of the liver, and spider angioma Eventual improvement is usually to

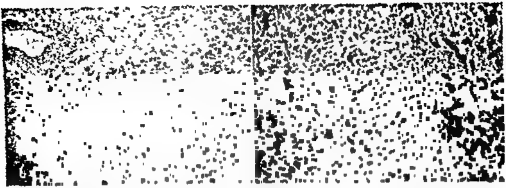

Fig 98 (*Left*) Photomicrograph (low power) of autopsy specimen, illustrating an acute destructive lesion of the liver from a case of infectious hepatitis in which death

of Dr G Klatskin)

be expected, although a small percentage of such patients have a progression of disease and develop severe postnecrotic scarring of the liver (Watson and Hoffbauer, 1946, Sherlock, 1948, Kunkel and Labby, 1950) In these, the ultimate prognosis is poor. Women in the age group beyond the menopause seem to be more prone to this form of chronic hepatitis than do men (Jersild, 1945).

Conditions that cause relapse or prolongation of disease are poorly defined It has been suggested that inadequate rest, poor diet, intercurrent infections, previous hepatic disease, and a long history of alcoholism or dietary insufficiencies may be predisposing factors, although little is known about these points

Pathologic Picture

Extrahepatic lesions found at necropsy include phlegmonous inflammation and hemorrhage of the stomach and the intestinal walls The spleen is frequently enlarged and congested with follicular hyperplasia Large edematous mesenteric and peripheral lymph nodes are found in patients dying in relatively early stages of the disease It seems that these rather scanty lesions fail to reflect the generalized character of this disease of which the hepatic lesion may be but one, albeit a spectacular, lesion Knowledge of pathologic changes in the liver has been acquired from biopsies of the liver taken at various stages throughout the course of the disease (Roholm and Iversen, 1939) and at necropsy (Fig 98). Early changes consist of swelling and irregularity of shape of hepatic

ration in most cases at the end of 2 or 3 months Slight residual periportal infiltration may persist in some patients for several months longer In the fulminant form of disease (Lucké and Mallory, 1946) in which death occurs within 10 days after onset, the

perplasia The lobular remnant contains numerous proliferated macrophages and erythrocytes, so that the liver may resemble a spongy framework infiltrated with inflammatory cells and blood. In such cases, death may occur very early, within 3 or 4 days of onset, before jaundice appears In the *subacute* form of disease in which death occurs 3 to 6 weeks after onset, the liver is frequently firm, reduced in size, and the cut surface may be granular. Destruction of parenchymal cells is neither complete nor uniform, and considerable evidence of regeneration is found

TABLE 24. COMPARISON OF CERTAIN FEATURES OF INFECTIOUS HEPATITIS AND SERUM HEPATITIS AND THEIR CAUSATIVE AGENTS, VIRUS A AND VIRUS B

CLINICAL AND EPIDEMIOLOGIC FEATURE	INFECTIOUS HEPATITIS	SERUM HEPATITIS
Incubation period	15 to 40 days	60 to 160 days
Type of onset	Acute	Insidious
Fever—over 38° C (100.4° F)	Common	Uncommon
Seasonal incidence	Autumn-winter	Year round
Age preference	Children and young adults	Any age
	Virus A	Virus B
Filtrability		
Seitz EK filter	Passed	Passed
Gradocol membrane with pore size 52 mμ	Not done	Passed
Susceptible host	Man	Man
Virus in feces	Incubation period and acute phase	Not demonstrated
Virus in duodenal contents	Acute phase	Not done
Virus in blood	3 days before onset and in acute phase	Incubation period and acute phase
Route of infection (experimental)	Oral and parenteral	Parenteral
Duration of carrier state,		
blood	As long as 8 months (one adult)	As long as 5 years (one adult)
feces	As long as 16 months (one child)	Not demonstrated
Immunity		
homologous	Present	Equivocal
heterologous	None apparent	None apparent
Prophylactic value of gamma globulin	Good	Equivocal

EXPERIMENTAL INFECTION; HOST RANGE

Various "Hepatitis Viruses"

In spite of numerous attempts to produce infectious (or serum) hepatitis in hosts other than man, these have been almost universally negative. A summary of these negative attempts as of 1949 was made by Colbert, and 2 years later in a British report (MacCallum et al., 1951), and again later in the United States in a report of a Symposium of the National Academy of Sciences—National Research Council (1954). These negative attempts have included the use of many different species of monkeys and chimpanzees. Occasional transmission of human hepatitis virus to pigs (Andersen and Tulinius, 1938), birds (Herzberg, 1943, Lucké and Radcliffe, 1949), embryonated eggs (Siede and Meding, 1941; Henle et al., 1950) and rats (MacCallum and Miles, 1946) has been claimed but not confirmed. More recently, ducks have been studied in this connection (Hanson and Alberts, 1956).

Various spontaneously appearing agents, some of which may or may not be related to human hepatitis virus, have been reported to be capable of producing hepatic lesions in sup-

TABLE 25. RESULTS OF EXPOSURE OF HEPATITIS VIRUSES A AND B TO VARIOUS PHYSICAL AND CHEMICAL AGENTS AS DETERMINED BY EXPERIMENTS IN VOLUNTEERS

	Virus A	Virus B
Temperature resistance		
Room temperature, 3 months	—	Survived
Room temperature, 8 months	—	Survived
56° C, 30 minutes	Survived	Survived
60° C, 1 hour	—	Survived
60° C, 2 hours	—	Survived
60° C, 4 hours	—	Survived
60° C, 10 hours (albumin)	—	Inactivated
−10 to −20° C, 1½ years	Survived	Survived
−10 to −20° C, 4½ years	—	Survived
Ultraviolet irradiation	—	Equivocal
Chlorine 1 p p m residual, 30 minutes	(1) Survived (2) Inactivated *	—
Tricresol 0 2%	—	Survived
Phenol-ether (equal parts) 0 5%	—	Survived
Ether 10%, 24 hours at 4° C	Survived	—
Triple ether extraction of serum	—	Survived
Merthiolate 1:2,000	—	Survived
Nitrogen mustard (500 mg /liter)	—	Survived
Sulfur-mustard (0 005 M final conc)	—	Inactivated
Beta-propiolactone (4 Gm /liter)	—	Inactivated

— Not done

* Inactivation followed adequate coagulation and settling of water

posedly healthy mice (Olitsky and Casals, 1945, Nelson, 1952, Hara et al, 1952, Lackey et al, 1953, Jordan and Mirick, 1955) In 1951, Gledhill and Andrewes described a rather special kind of hepatitis in mice which was subsequently shown by the same authors (cf Niven et al, 1952) to be a complex infection due to two agents acting together the mouse hepatitis virus and another parasite, *Eperythrozoon coccoides* It is also clear that there are several agents, including the viruses of lymphocytic choriomeningitis and ectromelia, that may cause murine hepatitis In dogs, agents that give rise to hepatitis include infectious canine hepatitis virus (Rubarth, 1945). Such viral agents seem to be characteristically limited in their host range Nothing is known about a relationship between these agents and those producing hepatitis in man

Henle et al (1950) reported the propagation of virus A in tissue culture and embryonated eggs After several transfers, the virus was reidentified by suggestive but not conclusive tests in volunteers (Drake et al, 1950) Others, who have claimed the growth of human hepatitis virus in the developing chick embryo, include Dresel et al (1943), Benda et al (1949), and Essen and Lembke (1949), but none of this work has been confirmed

ETIOLOGY

From experiments with volunteers, it has been shown that infectious hepatitis virus (virus A) has certain properties that are listed in Tables 24 and 25 The actual number of recognized properties is small because of the

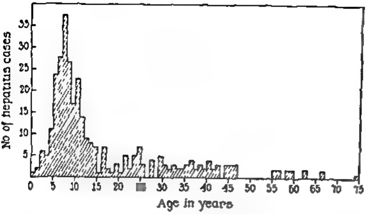

FIG. 99. The age incidence of *hepatitis epidemica* in Germany prior to World War II. Data are from two outbreaks occurring in Hamburg and Wilhelmsberg, respectively (von Bormann, F., 1940, Hepatitis epidemica, Ergebn. inn. Med u Kinderh *58, 201*)

obvious handicaps in working with this agent However, it passes through bacteria-tight filters and is transmissible in series to man; it is resistant to a temperature of 56° C for at least 30 minutes, and withstands chlorination for 30 minutes, viz, 1 part chlorine residual per million (Neefe et al, 1945a) as well as the action of certain chemicals that ordinarily destroy bacteria

Transmission

As far as is known, man continues to be the only original source of this agent, which is recoverable from the blood and the feces of patients in the incubation period as well as the preicteric or early icteric phase of disease and may be transmitted to volunteers by feeding or by parenteral inoculation of infectious material Several attempts to detect virus in the urine or the nasopharyngeal washings of patients at similar periods have yielded contradictory results (Findlay and Willcox, 1945) In general, they have been unsuccessful, and the balance of evidence is that intestinal excretion of this agent represents its main infective source

The period of infectivity of patients has been investigated (Havens, 1946a), but the number of experiments designed to determine when virus actually appears in the blood and the feces (presumably during the incubation period) and how long it remains there is unfortunately small However, it has been found in the blood 3 days before the onset of the disease (Francis et al, 1946) and also in the feces during the incubation period (Ward and Krugman, 1957). Attempts to recover virus A from the blood and the feces in adult patients 1 month after the onset of disease and also 3 weeks after disappearance of jaundice (Neefe et al, 1945c) have been unsuccessful However, Murray et al (1955a) found virus (presumably A) in the blood of 1 patient 8 months after recovery when tests of hepatic function were normal. This, in conjunction with the fact that a certain percentage of recipients of transfusions of whole blood acquire hepatitis after a short incubation period, suggests that the carrier state in the blood exists more frequently than previously suspected Infants can be fecal carriers of this virus for months (Capps et al, 1950). Further discussion of viremia and of hepatitis virus B is found in the section on *serum hepatitis*

Immune Reactions and Immunity

At present, the absence of specific diagnostic tests for the identification of the various types of human hepatitis viruses renders it most difficult to reach definitive conclusions about immunity. A number of serologic reactions have been described, but most of them have been accepted as nonspecific. A summary of these tests has been made by Havens (1954) Some of them are mentioned later.

Neefe et al. (1946) and Gauld (1946) suggest that one attack of infectious hepatitis is followed by homologous immunity The natural history of infectious hepatitis also supports the concept of a widespread adult immunity in some areas brought about by subclinical (and clinical) infection often acquired

in children and young adults than it is in

older groups (Fig 99). Among U S troops quartered in an endemic area of Europe in 1947-50, the morbidity rate in men of 20 years of age was about twice as great as that in men of 40 (Paul and Gardner, 1950).

The demonstration of the protective effect of normal human gamma globulin when administered during the incubation of infectious hepatitis suggests the presence of certain neutralizing substances in the blood of the normal human adult population, probably as a result of a previous clinical or subclinical attack of the disease Immunity has also been demonstrated experimentally (Neefe et al, 1945b, 1946; Havens, 1946b) in volunteers convalescent from hepatitis caused by 2 different strains of virus A when challenged with the homologous strain 6 to 9 months after their initial infection Partial cross-immunity was also shown to exist between these 2 strains of virus A A history of second attacks of hepatitis is said to be obtainable in from 2 to 5 per cent of patients Again, the lack of a specific diagnostic test makes it difficult to evaluate such evidence, for one cannot eliminate the possibility of prior infection due to virus B or to other as yet unidentified and immunologically distinct strains of hepatitis virus. The failure to obtain these viruses in a form in which their antigenic properties can be studied has been the main reason why information on this point is very incomplete

Diagnosis

In the absence of specific immunologic tests, diagnosis in the preicteric phase must be made on clinical and epidemiologic evidence Early in the disease, percussion tenderness over the liver and the detection of posterior cervical adenopathy and splenomegaly may be of assistance Abnormal retention of bromsulphalein is usually the first test of hepatic function to become positive, and this may occur as early as the second day of fever The cephalin-cholesterol flocculation and the thymol turbidity tests become positive somewhat later, and ordinarily bilirubin appears in the urine toward the end of the preicteric phase just before jaundice is apparent (Fig 97) The detection of bilirubinuria is a simple but reliable diagnostic aid at this time and may also be helpful in nonicteric hepatitis Its value has been emphasized by Swift et al (1950b). Leukopenia with relative lymphocytosis is characteristic in the preicteric phase and it simulates that seen in the early stages of infectious mononucleosis

The sera of a very few patients with infectious hepatitis contain a heterogenetic antibody that agglutinates sheep erythrocytes Some sera also contain antibodies that agglutinate rabbit erythrocytes and fix complement when combined with an antigen made from human liver. These tests have not turned out to be of diagnostic significance. Indeed, the absence of agglutinins for sheep cells has been a point of differentiation between the hepatitis of infectious mononucleosis and that of infectious or serum hepatitis

During the febrile preicteric phase, diseases that may be confused are infectious mono-

fluenza The subsequent course and the demonstration of specific etiologic agents or their antibodies in the case of these other diseases assist in making the diagnosis

When jaundice is present, the following conditions may be considered acute and subacute cholangitis, Weil's disease and yellow fever Jaundice may also develop occasionally in a variety of acute and chronic infections, as in infectious mononucleosis, malaria brucellosis, amebiasis, pneumococcic pneumonia, septicemias, syphilis, both congenital and acquired (secondary) In addition to jaundice associated with various infections, other types of jaundice to be distinguished include (1) hemolytic, either congenital or acquired, (2)

carcinoma of the liver, and (3) obstructive, due to obstruction of the biliary tract by calculus or neoplasm

A diagnosis of chronic viral hepatitis depends on the demonstration of hepatic dysfunction or histologic alterations in the liver, with or without clinical signs or symptoms Although the wide variability in the duration of illness does not justify this diagnosis until at least 6 months have elapsed from the onset of acute hepatitis, the reappearance and the persistence of anorexia, easy fatigability, and upper abdominal discomfort beyond this time

suggest chronic hepatitis. The presence of prolonged enlargement and tenderness of the liver, *jaundice and splenomegaly, and abnormal* tests of liver function lend supportive evidence, and in such patients abnormality of the bromsulphalein-retention test is the most reliable evidence of chronic hepatic dysfunction. Cholangiolar obstruction occurs at times, with severe jaundice and increased amounts of serum alkaline phosphatase and total cholesterol. Differential diagnosis from extrahepatic obstruction may be particularly difficult in such patients, and biopsy of the liver, or even exploratory laparotomy, may be indicated at times In a certain number of patients who have had acute hepatitis, it is most difficult to interpret their persistent subjective complaints in the absence of objective evidence of hepatic disease It is recognized that anorexia, fatigue and abdominal discomfort may occur long after the expected time of recovery when little or no evidence of hepatic dysfunction or histologic alterations in the liver is found; the term "posthepatitis syndrome" has been applied to this condition (Sherlock and Walshe, 1946).

Treatment

Acute Hepatitis. Treatment of the disease is symptomatic and, under the circumstances of normal civilian life, there is little need to hospitalize patients if they are not seriously ill The most important therapeutic principles are the provision of adequate rest in bed and a well-balanced, palatable, nutritious diet Procedures outlined by Chalmers et al (1955) are recommended They advocate an *optimal diet consisting of about 3,000 calories* and containing approximately 150 Gm each of protein and fat. Intakes above this level *should be ad lib. Although fried and greasy* foods may cause indigestion, the fat contained in meat, eggs and dairy products is not harmful and adds greatly to the palatability of the diet. During the stage of severe anorexia, the patient should be urged to take frequent small feedings. Intravenous glucose solutions should be administered when necessary to maintain a minimal caloric fluid intake. Although the forcing of a high-protein, high-fat diet, by stomach tube if necessary, in patients with disease of average severity has been demonstrated to hasten recovery, critically ill patients with fulminating disease or impending hepatic coma may be harmed by ex*cess dietary protein. Therefore,* in these few very ill patients it is probably unwise to administer more than a maintenance quantity of protein, and under certain circumstances protein should be completely omitted from the diet during the critical phase of illness Intra*venous protein hydrolysates, plasma* or blood transfusions have no place in the nutritional therapy of patients with uncomplicated infectious hepatitis The duration of rest in bed, which is recommended in this disease, has been a controversial matter. Military experience early in World War II indicated that too brief a period of bed rest in depleted patients with this disease was followed not infrequently by relapse. Subsequent experience with troops who were in good physical condition before contracting the disease (Gardner et al., 1949; Swift et al , 1950a) suggested [illegible]

Chalmers et al. (1955) have pointed out that physical activity to the point of fatigue may be harmful. Patients should be urged to rest *in bed as long as acute symptoms* persist Once they begin to feel well, regardless of the degree of jaundice, they should not be forced to stay in bed more than 1 hour after each meal. However, restriction to the room or the hospital ward is essential to decrease undue activity or exertion. Allowing this type of *ad lib* activity (without any required exertion) circumvents the usual delay necessary for recuperation from the effects of prolonged *rest in bed and appreciably* shortens the period of convalescence.

Patients so treated may begin physical re*conditioning after the total serum* bilirubin is below 1.5 mg. per 100 ml. and the bromsulphalein retention in 45 minutes is below 6 per cent for a period of not less than 1 week Patients whose bromsulphalein retention stabilizes between 5 and 10 per cent may begin physical reconditioning with safety. Those with persistently higher levels will require individual management. During the period of reconditioning, it is well to follow all patients with weekly physical examinations, serum bilirubin tests and bromsulphalein tests until convalescence is complete—usually a matter

of 2 to 4 weeks Recurrent abnormalities will occur rarely and are probably indication for diminishing activity In the average adult military case with jaundice, the period of hospitalization is 60 days.

The administration of corticosteroids or ACTH in the acute phase of viral hepatitis is usually followed by a speedy return of a sense of well-being and appetite, a sharp diminution in serum bilirubin within 1 week after institution of therapy, and a shortening of the total period of jaundice in amount of 1 week (Colbert et al, 1951, Ducci and Katz, 1955) However, untoward effects, including "mooning" of the face, ascites, edema and hypertension, are frequently observed, and relapse following cessation of too short a course of therapy is not uncommon (Evans et al, 1953) For these reasons, the routine use of ACTH or corticosteroids is not recommended in patients with hepatitis of average severity Nevertheless, there are situations in which special consideration for therapy with corticosteroids or ACTH must be given (Nelson, 1957) These include (1) patients with severe progressive disease, intractable vomiting, and with serum bilirubin in excess of 15 mg per 100 ml., (2) patients with disease of such severity that hepatic coma is imminent or present, and (3) patients with cholangiolitic hepatitis

When coma occurs in the course of acute hepatitis, recovery is rare In such situations, Ducci and Katz (1952) first showed that cortisone used in conjunction with other measures ordinarily employed for treating such patients may have beneficial effects These workers and others, using a regimen of 500 mg cortisone injected intramuscularly every 8 hours for 1 to 2 days, followed by rapidly diminishing doses over the next 14 to 21 days, have reported survival of a small number of patients In such patients it is also recommended (1) that chloramphenicol be given (Farquhar et al, 1950; Shaffer et al, 1950) in amounts of 250 mg every 6 hours by mouth, supplemented by 100 mg every 6 hours intravenously, and (2) that from 2,000 to 3,000 ml of a solution of 10 per cent dextrose in sterile distilled water be given intravenously in constant drip daily during the acute phase of hepatic decompensation.

Chronic Hepatitis. *In certain patients with mild though continued functional hepatic disability as the only manifestation of sequelae of acute hepatitis, it would seem unnecessary to curtail their activity for any therapeutic reasons. However, another group of patients with symptoms and signs of disease, including prolonged anorexia, epigastric discomfort (with or without jaundice), and an enlarged, tender liver, may require rest in bed for prolonged periods, with special attention to a well-balanced, nutritious diet. In those patients whose disease progresses to severe postnecrotic scarring of the liver, the usual therapeutic regimen employed for persons with Laennec's cirrhosis is recommended but is frequently disappointing The intravenous administration of salt-poor albumin appears to be of some value in certain individual cases of this type*

Epidemiology

Prevalence and Geographic Distribution. In spite of inadequate information, so-called viral hepatitis probably occurs in nearly all inhabited areas of the globe. However, there are certain regions with an established record of high endemic prevalence for infectious hepatitis,* of which the Middle East and the Mediterranean littoral are examples Military outbreaks are proverbial there, and over the past 150 years these have occurred repeatedly among troops from Europe, Australia and North America which have been brought into such endemic areas

porting of cases during the past decade has been irregular and often on a voluntary basis It would be difficult to say what the relative incidence rates in this country by states might be. That it is a relatively common disease throughout the nation few would deny In Scandinavian countries, long-term information on the incidence of hepatitis has been more nearly complete than elsewhere This disease has been notifiable in Denmark since 1928 (Fig. 100) Here, as in some other countries in temperate climates in which sanitary facilities are good, the incidence has varied, with waves of increased frequency

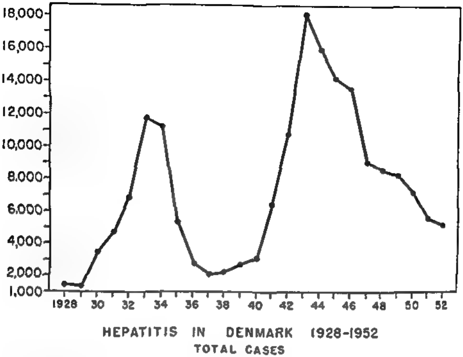

FIG 100. (From Marie Lindhardt, Director, Statistical Section, National Health Service of Denmark, Copenhagen, Denmark)

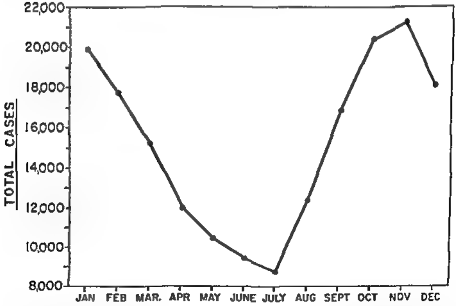

FIG 101. Seasonal trend of hepatitis in Denmark, 1928-1952. (From Marie Lindhardt, Director, Statistical Section, National Health Service of Denmark, Copenhagen, Denmark)

covering several years, notably during the occupation by German troops. How representative of northern Europe this picture may be is not known. Certainly, it is unlikely that the trends in Scandinavia would be similar to those of a tropical country or to those of a country with substandard conditions of sanitation.

In many areas there is a distinct seasonal trend, with a sudden increase in prevalence in the late summer and autumn, often building up to epidemic proportions during early winter and soon declining. However, the epidemic season may reach a peak in late winter and extend well into the spring (Fig. 101).

Transmission. Most present evidence indicates that infectious hepatitis is usually spread through some form of person-to-person association, although water-borne, food-borne and milk-borne epidemics have been described. Nevertheless, man represents the main source and reservoir of the infection, for as yet no extrahuman host in the form of an animal, a bird or an arthropod has been discovered in which hepatitis viruses are known to multiply or survive, although the possibility of mechanical transmission by a biting insect never has been eliminated nor has it been investigated adequately.

There is good evidence to indicate that the intestinal-oral circuit is the principal route in this natural person-to-person spread. This is based upon experimental tests with volunteers that indicate the frequency with which hepatitis virus A can be demonstrated in human feces during acute stages of the disease, and the ease with which this disease may be produced by feeding such material to man. Although some epidemiologic observations point to a respiratory mode of spread, none of the experimental tests on this has been sufficiently satisfactory (due to technical or other reasons) for definite conclusions to be drawn from the results. In any event, a respiratory type of spread seems unlikely. As in poliomyelitis, personal and close contact with infective people, particularly children, probably provides the source of exposure for a great majority of orally acquired cases of infectious hepatitis.

Evidence to substantiate the fact that the intestinal-oral circuit is involved is based upon the water-borne epidemics of infectious hepatitis and the [illegible]

of a variety of enteric infections. Not infrequently outbreaks of hepatitis are preceded by a wave of "gastroenteric illness," presumably of bacterial origin. An example of this has been reported by Tucker et al. (1954) in the form of water-borne gastroenteritis and hepatitis epidemics that sequentially occurred at a church camp in Tennessee in 1952. It was suggested here, as elsewhere, that both infections, acute gastroenteritis and infectious hepatitis, were related to the same source, and the difference in the lengths of the respective incubation periods (i.e., a few days in the former and about 3 weeks in the latter) gave rise to 2 sequential waves of illness. Military observations also record that the incidence of hepatitis has been highest when and where the sanitation of camps has been poor. Civilian evidence further testifies to the frequency with which visitors acquire hepatitis in tropical or semitropical areas, and also where poor sanitary facilities exist. Again, in institutions for mentally deficient individuals, hepatitis rates have been higher in those buildings housing individuals with the lowest mental capacity and where, as a consequence, the poorest sanitation can be maintained.

Nevertheless, as viral hepatitis is easily spread by intimate association among people, epidemics of this disease commonly result from some form of person-to-person dissemination, within institutions, dwelling houses or apartment houses where sanitary facilities are far from substandard. Such outbreaks often occur in the best-regulated families. Within some "communities," it may well be that a large unsuspected source of virus maintains itself in the form of inapparent, infantile or juvenile cases, and under circumstances of unsuspected exposure it is small wonder that the disease spreads readily to the susceptibles in the population.

The artificial parenteral dissemination of virus A, in a manner comparable with that usually seen with serum hepatitis (virus B), through the use of contaminated blood or serum, and by improperly sterilized syringes or needles as described by Droller (1945), is a point for consideration. The stability of the icterogenic agent and the infectivity of small quantities of blood suggest that infectious hepatitis probably has been transmitted unintentionally more often than is realized.

Types of Epidemics (1) *Water-borne.* There are now many examples of the explosive water-borne epidemic. Prominent among them have been those reported in summer

camps (Neefe and Stokes, 1945, Tucker et al, 1954) A notable recent example of a huge water-borne urban epidemic of infectious hepatitis is one that occurred in 1956 in the city of Delhi, India (Melnick, 1957) It is said to have accounted for some 30,000 cases (2) *Food-borne and Food Handlers* Food-borne (including milk-borne) epidemics have been described in military and civilian experience (Read et al, 1946; Murphy et al, 1946), and of particular interest was an epidemic apparently resulting from eating contaminated oysters (Gard and Alin, 1957) It is more than likely that food handlers in various infectious stages of inapparent or clinical hepatitis play an important role in the spread of virus A

Institutional Outbreaks. These are common in orphan asylums, boarding schools, and notoriously so in mental institutions In some large mental institutions (i e., with a population of 2,000 or more), hepatitis and salmonellosis may become endemic, and over the course of several years cases may continue to appear in large or small numbers each month both in inmates and in attendants (Ward et al, 1957) This situation has been ascribed to the bowel habits of mentally deficient inmates, be they children or adults, and to the great difficulties of maintaining adequate standards of environmental sanitation even in the best-run institutions

Family Epidemics. These are so common and well known as hardly to deserve mention In a family consisting of 4 or 5 members, a usual story is that 1 child contracts a mild case of "jaundice" first, and not much is made of it. About a month later, another child becomes jaundiced or perhaps a parent Usually much more attention is paid to the case occurring in an adult, although to the epidemiologist the others would seem to be of equal importance The rather long interval between cases offers an opportunity to use gamma globulin as a prophylactic measure in such situations

Age Distribution. Under most circumstances, infectious hepatitis is essentially a disease of childhood Indeed, as a general statement, children are said to account for 65 per cent of the cases, with the bulk of them occurring between the ages of 5 and 15 (cf. Fig 99). It is obvious, as has already been mentioned, that this distribution may vary considerably under different environmental conditions, in different places or at different times in the same place This concentration of the disease in juveniles has been responsible for many misconceptions regarding the local incidence of viral hepatitis In endemic areas, infection and immunity are acquired so early in life and at an age when the clinical symptoms are about at the vanishing point that they may be completely overlooked Mild diarrhea may be the only symptom Superficially, therefore, the disease often seems to be conspicuous by its absence Only does its presence come to light when susceptible visitors enter the endemic area

This mildness of infantile and childhood hepatitis has not received the emphasis it deserves, particularly as there is no evidence that such mild cases are any the less infectious, indeed, as the duration of the intestinal carrier state in infants is so prolonged, they may be more infectious In this sense, these long-term infant carriers (Capps et al, 1950) can actually serve as a reservoir of infection

Control Measures

The reporting of all cases (including suspected cases) is a useful measure, and in some places it is required For the protection of intimately exposed persons, the prompt administration of normal immune globulin has proved to be a valuable procedure, particularly in the case of the adult members of a household exposed to infectious hepatitis Illness may be aborted if immune globulin is given during the incubation period or perhaps just before exposure It may be effective when given as late as 6 days before the onset of disease (Stokes and Neefe, 1945, Havens and Paul, 1945, Gellis et al, 1945) Doses of 0 06 to 0 12 cc. per pound of body weight are protective when given intramuscularly, although it has been shown that as little as 0 01 cc per pound of body weight is effective It has been estimated that passive protection lasts from 6 to 8 weeks However, observations of groups of children inoculated with gamma globulin and subsequently exposed to hepatitis virus under endemic conditions over a period of several months suggest that more permanent protection may occur. The reason for this is not yet understood (Stokes et al, 1951).

As for general methods to curtail the spread of virus A, procedures that tend to interrupt the intestinal-oral route should be carried out During any institutional or camp outbreak, attention should be directed toward the gen-

eral sanitation of the site and of the water supply, fly abatement, sterilization of food receptacles, and prevention of fecal contamination of food and milk supplies The degree of chlorination effective against hepatitis virus has not yet been determined Detection of healthy human carriers or subclinical cases is impossible at present because of the lack of suitable laboratory technics Since it is as yet unknown how long virus remains in stools or blood, it is advisable to regard the stools as potentially infectious for at least 1 month after the onset of disease and to recommend that patients do not act as donors of blood except under the special conditions described in the section on serum hepatitis Every possible effort should be made to avoid employing food handlers who may be fecal carriers of the virus and to keep food handlers free from infection Particular effort should be made to clean properly and to sterilize with heat all needles and syringes that come in contact with the blood of such patients There will be further discussion of this important point in the section on serum hepatitis

SERUM HEPATITIS

Introduction

Serum hepatitis is described as a form of disease ordinarily produced by the parenteral inoculation of human blood or its products obtained from a person who, though as a rule not apparently ill, is carrying virus B in his blood The fact that virus A is also carried in the blood and may be transmitted parenterally limits the usefulness of this definition, and at present it is generally assumed that if hepatitis acquired in this way has a short incubation period (15 to 40 days), it is the result of the artificial transmission of virus A, whereas a long incubation period (60 to 160 days) implicates virus B as the cause of disease The recent description by Ward et al (1957) of epidemic hepatitis (presumably due to virus A) with an intermediate incubation period of 35 to 50 days and the fact that a fair percentage of patients acquiring hepatitis from transfusions of whole blood have incubation periods of a similar length emphasize the inadequacy of nomenclature based on length of incubation period

History

Under the terms of this arbitrary definition, it is likely that this type of hepatitis has existed for some time, its incidence augmented during recent years by the increasing use of human blood and certain of its products and by the frequency of various parenteral penetrations The epidemics of jaundice following vaccination for smallpox in Germany, Lurman, 1885, in the 1880's were doubtless examples of this disease. Likewise, the early suggestions of Stokes et al, 1920, and Ruge, 1927, that the jaundice occurring in luetic patients receiving heavy-metal therapy was due to an infectious agent were probably correct. However, credit for the first recognition of the true nature of the condition goes to a group of Swedish physicians (Flaum et al, 1926) who described an

atitis may be divided into two groups (1) those who are infected by improperly sterilized syringes, needles or instruments employed in giving medications, withdrawing blood for laboratory tests, or performing various procedures in dental work or even tattooing, and (2) those who are infected by the administration of transfusions of blood or plasma, or contaminated products of human blood such as convalescent serum, dried serum, fibrinogen, thrombin, Cohn's Fraction IV, or vaccines containing human serum or plasma In the years just prior to and early in World War II, thousands of cases of serum hepatitis occurred, particularly in military personnel but also in civilians, after the injection of yellow fever vaccine that contained human serum (Fox et al, 1942, Sawyer et al, 1944) Subsequently, the widespread use of plasma and blood particularly in depleted patients both military and civilian, together with the failure to develop satisfactory methods of sterilizing these materials to render them free from hepatitis virus, and the frequency and the prolonged duration of the carrier state augmented the seriousness of this hazard to the point where it has emerged as one of the important medical problems of the times The potential magnitude of risk is attested by the fact that 21 9 per cent of certain groups of soldiers receiving transfusions of blood and plasma and 3 6 per cent of other groups receiving

whole blood alone in the Korean War contracted serum hepatitis (Sborov et al, 1953) Among civilians, the incidence has not reached these proportions, although rates as high as 11 9 per cent have been reported following the use of plasma from large pools (Murphy and Workman, 1953) In addition, the protracted disruption of civilian economy during the postwar years, with short supplies in many parts of the world, created situations favorable to inadequate sterilization of instruments and to the artificial transmission of hepatitis virus in clinics and physicians' offices (Wewalka, 1953)

Clinical Picture

With certain exceptions, serum hepatitis is clinically and pathologically similar to infectious hepatitis However, its incubation period is much longer, and its onset is more apt to be insidious and accompanied by less fever (Turner et al, 1944) Worthy of mention also is the frequency with which urticaria and arthralgia precede the appearance of jaundice The clinical course is usually similar in severity to that of infectious hepatitis when otherwise healthy patients in the same age groups are compared, however, serum hepatitis, by the nature of its way of transmission, so frequently occurs in sick, debilitated and often elderly patients that in them as well as in young children (McNalty, 1938) its course may be much more severe and the mortality greater The same therapeutic principles apply to both conditions

Etiology

The little that is known about the properties of virus B has also been derived from experiments in volunteers Its exact size is not defined, but it passes through a gradocol membrane of 52 mμ average pore diameter, indicating a size of 26 mμ or less (McCollum, 1952) It is also highly resistant to inactivation by various physical and chemical agents; is transmissible to man in series; and, under certain circumstances, evokes homologous immunity, although little is known about this response and its status is equivocal (Table 24)

Transmission

Man is apparently the only original source of this agent, which is recoverable from the blood of carriers and from patients during the long incubation period or early icteric phase of disease and may be transmitted to volunteers by parenteral inoculation of infectious material Limited attempts to detect virus in the feces, the urine and the nasopharyngeal washings (with one exception—Findlay and Martin, 1943) have been unsuccessful, and the available evidence suggests that the blood of human carriers represents its main infective source.

The duration of infectivity following recognized hepatitis is unknown A small number of earlier studies with experimentally infected volunteers suggested that virus A and virus B were not present in the blood when tested at varying times ranging from 1 to 12 months after the onset of disease However, subsequent studies revealed the presence of virus B in the blood of one patient 4 months after recovery and of virus A in the blood of another patient 8 months after recovery. Tests of hepatic function were normal at the times when viremia was present (Murray et al, 1955a) Of greater importance, however, is the accumulated evidence indicating that the carrier state without recognized disease is well equilibrated under certain circumstances and exists more frequently than previously suspected The prolonged period of viremia during the incubation periods of experimentally infected volunteers who were asymptomatic and without laboratory evidence of hepatic dysfunction is in support of this concept, as is the fact that, up to the present, those persons proved to be carriers have not, with a few exceptions, had a history of preceding hepatitis or jaundice. Clinical observations (Stokes et al, 1954) furnish supportive evidence in that virus B was found on two occasions, 2 years apart, in the blood of a patient who was asymptomatic and had normal hepatic function as determined by tests. During this period, the patient was delivered of a baby that died of hepatitis Another patient with so-called Laennec's cirrhosis has been proved to be a carrier of virus B for a period of 5½ years Lastly, the development of hepatitis by donors as long as several months to 1 year after their blood had been suspected or proved to have caused the disease in more than one recipient attests the equilibration that exists in

the carrier state (Murray et al, 1955a, Havens, 1956b)

Immune Reactions and Immunity

The nature and the degree of the immune response in serum hepatitis are poorly defined, since there is no method of measuring it at present. The fact that a large percentage of persons inoculated with virus B, either as volunteers or as patients receiving contaminated transfusions of blood or plasma, fail to contract hepatitis suggests either inherent or acquired resistance in the recipients, and the latter possibility is supported by the demonstration of homologous immunity in some of the studies in volunteers (Neefe et al, 1946; Murray, 1954) However, in contrast are (1) the failure to prevent infection with virus B in volunteers simultaneously inoculated with virus and with homologous convalescent gamma globulin (Drake et al, 1953); and (2) the irregular results of attempts to prevent serum hepatitis in field trials with different lots of normal human gamma globulin (Grossman et al, 1945, Duncan et al, 1947, Mirick, 1957) Of interest in this regard is the fact that infections with virus B, in contrast with those with virus A, apparently do not have a predilection for any period of life; all age groups appear to be susceptible It is not known whether the variability in protection against virus B is due to (1) insufficient exposure to virus because of the artificial way of transmission, (2) the failure to develop or maintain adequate immunity following exposure, or (3) the existence of multiple strains of virus that are immunologically unrelated or have a limited geographic distribution These questions are particularly pertinent when, as occasionally happens, multiple attacks of hepatitis occur in the same patient (Fig 102, Havens, 1956a)

Epidemiology

The geographic distribution of serum hepa-

d together in time and

tis, strains of virus (presumably B) have been found that produced hepatitis in volunteers with a long incubation period following par-

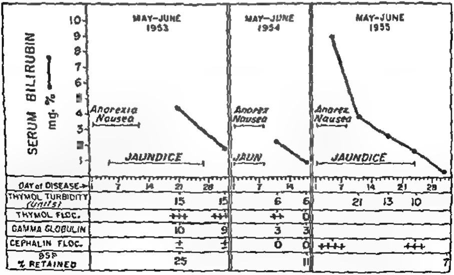

Fig 102. Course of disease during 3 separate attacks of viral hepatitis that occurred at intervals of 1 year in a narcotic addict (Havens, W. P, Jr., 1956, Viral hepatitis. multiple attacks in a narcotic addict, Ann Int. Med 44, 199-203)

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is warranted even though it is only partially effective and at considerable sacrifice of blood.

Both virus A and virus B are unusually resistant to inactivation by physical and chemical agents, and there is no practicable way to treat all products of human blood to render them safe (Table 25) In this regard, it should be emphasized that there is no knowledge as to whether such commonly employed disinfectants as alcohol, ether and Zephiran are effective in destroying either virus A or virus B Accordingly, the probable inadequacy of disinfectants or detergents for so-called "cold sterilization" of surgical and dental instruments should be considered in this light. More is known of the resistance of virus B because of the numerous attempts that have been made to sterilize whole blood or plasma of this virus, utilizing ultraviolet light, heat, nitrogen mustard, sulfur-mustard, beta-propiolactone, and storage at room temperature for varying periods None of these methods has been proved to be effective consistently, although the evidence obtained experimentally and by follow-up studies of patients receiving plasma suggests that the use of one or more of them probably inactivates virus in some degree

Follow-up reports on patients receiving plasma believed to have been rendered safe by ultraviolet irradiation revealed an incidence of hepatitis as high as 11 9 per cent in one group of recipients (Murphy and Workman, 1953) Other studies by Murray et al (1955b) with a variety of technics revealed that irradiation as performed at present is not effective on a production basis or at levels of intensity that do not alter plasma proteins It is noteworthy, however, that under the conditions of these studies there apparently was some vitiating effect on virus B, since the volunteers inoculated with it had a lengthening of the incubation period and a milder disease

The greatly diminished incidence, or even absence, of hepatitis as determined by follow-up studies of recipients of plasma stored at room temperature for 6 months prompted Allen et al (1954) to propose this as a method of diminishing the hazard Experimentally, Murray et al (1954b) showed that known icterogenic plasma (containing virus B) stored at 70° F for 3 and 6 months was still infectious for volunteers, although the results indicated that the infectivity had been sharply reduced by storage for 6 months However, there are inherent limitations to this procedure, including the possibility of bacterial contamination and alterations of plasma proteins In addition, the necessity of waiting 6 months for inactivation limits the practicality of this method if rapid stockpiling of plasma were required

Although there is no single method of inactivating hepatitis virus that is applicable to human blood and all its components, ways of treating individual fractions have been devised that appear to be effective. The present method of preparing normal serum albumin includes heating to 60° C. for 10 hours, and this treatment has been shown to inactivate virus B when it is added to such preparations (Gellis et al, 1948) In addition, immune serum globulin and serum albumin prepared from known icterogenic plasma (containing virus B) by cold-ethanol fractionation, with subsequent heating of the albumin to 60° C for 10 hours, were not infectious in volunteers (Murray et al, 1955a) These results confirm the widely held clinical beliefs that neither immune serum globulin (Janeway, 1945, Hammon et al, 1952) nor normal serum albumin (Paine and Janeway, 1952) can be incriminated as causing serum hepatitis. However, of interest in this regard are recent observations indicating that virus B may not be completely removed when immune globulin is prepared (1) by ether fractionation (Cockburn et al, 1951) or (2) by zinc precipitation, nor does the latter step completely remove virus B from stable purified protein solution (S P P S unheated) (Murray et al, 1955a) Of importance also are the facts that (1) virus B withstands heating to 60° C for 4 hours in plasma (Murray and Diefenbach, 1953), and (2) attempts to inactivate it in whole blood by the addition of nitrogen mustard were unsuccessful (Drake et al, 1952) Preliminary experiments with sulfur-mustard and beta-propiolactone indicate that both of these materials can inactivate virus B, although the exact conditions for use and ultimate possibilities are not yet defined (Murray et al, 1955a).

Because of the carrier rate, the high degree of resistance of virus A and virus B to

enteral inoculation (Oliphant et al, 1943; Paul et al, 1945; Evans, 1950)

Comparison of Serum Hepatitis and Infectious Hepatitis

Certain differences are apparent between these 2 forms of hepatitis and their causative viruses (Table 24). Virus B is present in the circulating blood during the long incubation period as well as in the active stage of disease (Havens, 1946c), indeed, it has been demonstrated (Neefe et al, 1944) in the blood 87 days prior to the onset of symptoms It apparently produces disease only when inoculated parenterally (one exception *reported by MacCallum and Bauer, 1944), and symptoms appear insidiously after a long and variable incubation period of from 60 to* 160 days The disease is not so contagious as *infectious hepatitis, infection by close* contact has been suspected but is rare, and virus B has not been demonstrated in the feces as virus A has been in patients with infectious hepatitis These facts, together with the failure to produce hepatitis in volunteers by the oral administration of serum known to contain virus B, suggest that the mechanism whereby serum hepatitis is acquired in nature is obscure Immunologically, virus A and virus B appear to be distinct Studies on immunity in volunteers corroborate the epidemiologic experience that patients who have had infectious hepatitis are susceptible to serum hepatitis, and vice versa (Havens, *1945b, Neefe et al., 1945c) However, it is* not yet known whether this distinction represents actually *different viruses or antigeni*cally dissimilar strains of a single virus The curious apparent dependence of virus B on artificial transmission, its long incubation period, and the contradictory evidence of immunity following infection with it raise the question that the family that goes to make up virus B may represent variants of virus A, which is normally transmitted through the intestinal-oral route, produces disease after a short incubation period, and evokes a fairly solid immunity *in man The geographic co*existence of virus A and virus B, their im*munologic* distinctions, and the fact that both may be transmitted parenterally have sug*gested* that under certain circumstances both agents might be transmitted within a short interval or *even simultaneously, thereby* explaining the wide variations in length of incubation periods *sometimes seen during* localized outbreaks or even the occurrence of two attacks of disease (with a short and a long incubation period) in the same person (MacCallum, 1953, Wewalka, 1953).

Prevention and Control

Attention has been called to the fact that the emergence of serum hepatitis as a tremendous problem during the past 20 years occurred as a result of (1) the increasingly widespread use of human blood and its products; (2) the failure to develop reliable methods of sterilizing most of these materials of hepatitis virus, (3) the inadequate cleansing and sterilization of various instruments; (4) the frequency and the prolonged duration of *the carrier state. It has been estimated that* as many as 0.5 per cent of persons may be *carriers of virus under certain circumstances* The well-established role of the carrier state in *maintaining this infection, together with* the evidence suggesting that virus B may be transmitted from mother to child in utero, may account in part for its survival in nature at present (Stokes et al, 1954; Neefe et al, 1954, Murray et al, 1954a)

It is difficult to devise measures for the control of this disease because of the lack of specific serologic tests, susceptible laboratory animals and accurate methods for the detection of carriers The use of various tests of hepatic function (*particularly the thymol* turbidity) to detect carriers of virus may reveal *evidence of hepatic disease* and, in retrospect, has furnished evidence that certain donors whose blood produced hepatitis actually had hepatic disease Unfortunately, as many as 15 per cent of some groups of presumably normal persons have abnormal thymol turbidity tests The disproportion between this and the estimated carrier rate (up to 0 5%) is viewed with concern by those responsible *for blood banks* and makes *them* reluctant to reject so many donors on this *basis (Murray, 1955). In addition, the* fact that asymptomatic donors, with normal hepatic function as determined by tests, have been proved to be carriers of virus makes this an unreliable method of detection However, further consideration of this safeguard

Essen, K W, and Lembke, A., 1949, Zur Ätiologie der Hepatitis epidemica, Ztschr klin Med *145*, 517-533

Evans, A S, 1950, Serum hepatitis in U S troops in Germany, Proc Soc Exper Biol & Med *75*, 809-811

Evans, A S, Sprinz, H., and Nelson, R S, 1953, Adrenal hormone therapy in viral hepatitis II The effect of cortisone in the acute disease Ann Int Med *38*, 1134-1147

Farquhar, J D, Stokes, J, Jr, Whitlock, C M, Jr., Bluemle, L W, Jr., and Gambescia, J M, 1950, Studies on the use of aureomycin in hepatic disease III A note on aureomycin therapy in hepatic coma Am J M Sc *220*, 166-172

Findlay, G M, MacCallum, F O, and Murgatroyd, F., 1939, Observations bearing on the aetiology of infective hepatitis (so-called epidemic catarrhal jaundice), Tr Roy Soc Trop Med & Hyg *32*, 575-586

Findlay, G M, and Martin, N H, 1943, Jaundice following yellow fever immunization Transmission by intranasal instillation Lancet *1*, 678 680

Findlay, G M, and Willcox, R R., 1945, Transmission of infective hepatitis by faeces and urine, Lancet *1*, 212

Flaum, A, Malmros, H, and Persson, E, 1926, Eine nosocomiale Ikterus-Epidemie Acta med scandinav, Suppl 16

Fox, J P, Manso, C, Penna, H A, and Pará, M, 1942, Observations on the occurrence of icterus in Brazil following vaccination against yellow fever, Am J Hyg *36*, 68-116

Francis, T, Jr, Frisch, A W, and Quilligan, J J, Jr, 1946, Demonstration of infectious hepatitis virus in presymptomatic period after transfer by transfusion, Proc Soc Exper Biol & Med *61*, 276-280

Gard, S, and Alin, K, 1957, Studies on the hepatitis virus *in* Hepatitis Frontiers, p 169, edited by Hartman, F W, et al, Boston, Little

Gardner, H T, Rovelstad, R A, Moore, D J, Streitfeld, F A, and Knowlton, M, 1949, Hepatitis among American occupation troops in Germany *a follow-up study with particular reference* to interim alcohol and physical activity, Ann Int Med *34*, 1009-1019

Gauld, R L, 1946, Epidemiological field studies of infectious hepatitis in the Mediterranean Theatre of Operations I-VIII Am J Hyg *43*, 248-313

Gellis, S S, Neefe, J R, Stokes, J, Jr, Strong, L E, Janeway, C A, and Scatchard G, 1948, Chemical, clinical, and immunological studies on the products of human plasma fractionation XXXVI Inactivation of the virus of homologous serum hepatitis in solutions of normal human serum albumin by means of heat J Clin Invest *27*, 239-244

Gellis, S S, Stokes, J, Jr, Brother, G M, Hall, W M, Gilmore, H R, Beyer, E, and Morrissey, R A, 1945, The use of human immune serum globulin (gamma globulin) in infectious (epidemic) hepatitis in the Mediterranean Theatre of Operations I Studies on prophylaxis in two epidemics of infectious hepatitis J.A.M.A *128*, 1062-1063

Gledhill, A W, and Andrewes, C H, 1951, A hepatitis virus of mice, Brit J Exper Path *32*, 559-568

Grossman, E B, Stewart, S G., and Stokes, J., Jr., 1945, Post-transfusion hepatitis in battle casualties and a study of its prophylaxis by means of human immune serum globulin, J.A.M.A *129*, 991-994

Hammon, W M, Corell, L L., and Stokes, J., Jr., 1952, Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis I Plan of controlled field tests and results of 1951 pilot study in Utah J.A.M.A *150*, 739-749

Hanson, L E, and Alberts, J O., 1956, Virus hepatitis in ducklings, J Am Vet M A *128*, 37-38

Hara, K, et al., 1952, Studies on a virus isolated from a severe case of epidemic hepatitis, a probable causative agent of epidemic hepatitis Proc Japan Acad *28*, 270-276

Havens, W P., Jr, 1945a, Properties of the etiologic agent of infectious hepatitis, Proc Soc Exper Biol & Med *58*, 203-204

———, 1945b, Experiment in cross immunity between infectious hepatitis and homologous serum jaundice, Proc Soc Exper Biol & Med *59*, 148-150

———, 1946a, Period of infectivity of patients with experimentally induced infectious hepatitis, J Exper Med *83*, 251-258

———, 1946b, Immunity in experimentally induced infectious hepatitis, J Exper Med *84*, 403-406

———, 1946c, Period of infectivity of patients with homologous serum jaundice and routes of infection in this disease, J Exper Med *83*, 441-447

———, 1954, Review of attempts to test for immune bodies produced against the hepatitis agent *in* Symposium on the Laboratory Propagation and Detection of the Agent of Hepatitis, Publication 322, pp 93 99, National Academy of Sciences—National Research Council, Washington

———, 1956a, Viral hepatitis multiple attacks in a narcotic addict, Ann Int Med *44*, 199-203

———, 1956b, Unpublished observation

Havens, W P, Jr, and Marck, R E, 1946, The leukocyte response of patients with experimentally induced infectious hepatitis, Am J M Sc *212*, 129-138

Havens, W P, Jr, and Paul, J R, 1945, Prevention of infectious hepatitis with gamma globulin, J.A.M.A *129*, 270 272

Havens, W P, Jr, Ward, R, Drill, V A, and Paul, J R, 1944, Experimental production of hepatitis by feeding icterogenic materials, Proc Soc Exper Biol & Med *57*, 206 208

Henle, W, Harris, S, Henle, G, Harris, T N, Drake, M E, Mangold, F, and Stokes, J, Jr, 1950, Studies on the agent of infectious hepatitis I Propagation of the agent in tissue culture and in the embryonated hen's egg J Exper Med *92*, 271-281

Herzberg, K., 1943, Der Kanarienvogel als Versuchstier in der Hepatitis contagiosa-forschung, Klin Wchnschr *22*, 676 677

Horstmann, D M, Havens, W P., Jr, and Deutsch,

physical and chemical agents, and the extreme infectivity (0 01 cc. of serum is infectious), the following precautions are recommended. Every parenteral penetration should be performed with instruments that have been thoroughly washed and then sterilized by boiling in water for 30 minutes, or by autoclaving at 121° C (15 lbs. pressure) for 20 minutes, or with dry heat (180° C for 1 hour) Donors with a history of hepatitis, donors with close contact with patients with hepatitis during the preceding 6 months, or donors who have received a transfusion of blood or plasma during the past 6 months should not be accepted except under conditions of emergency for single transfusions or under conditions in which their blood may be used in the preparation of a product such as albumin, which can be sterilized of hepatitis virus The need for transfusion of whole blood or plasma should be carefully evaluated in each patient and, in particular, the use of plasma should be sharply limited If necessary, plasma should be made from blood obtained from not more than 2 donors (World Health Report, 1953, Report New York Academy of Medicine, 1957)

Specifically, attempts to prevent serum hepatitis by the administration of normal human immune globulin have had equivocal results and, in general, have been regarded as unsuccessful However, the results of administration of 2 doses of 10 ml each 1 month apart, with the first dose given shortly after the transfusion, suggest that some protection may be possible (Grossman et al, 1945, Mirick, 1957). In addition, since a goodly percentage of hepatitis following transfusion of whole blood has a short incubation period, presumably due to virus A, one would be justified in giving normal immune globulin (0 06 cc /lb) intramuscularly within 1 week after transfusion, repeating this dosage in 1 month, if sufficient supply of this material were available.

REFERENCES

(For complete references, see also 2nd edition, Chapter 35)

Allen, J G, Enerson, D M, Barron, E S G, and Sykes, C, 1954, Pooled plasma with little or no risk of homologous serum jaundice, J A M A *154*, 103-107.

Andersen, T T, and Tulinius, S, 1938, Etiology of hepatitis epidemica (epidemic jaundice), Acta med scandinav *95*, 497-509

Barker, M H, Capps, R B, and Allen, F. W, 1945, Acute infectious hepatitis in the Mediterranean Theater, J A M A *128*, 997-1003

Benda, L, Gerlach, F, Rissel, E, and Thaler, H., 1949, Über Untersuchungen zur Frage der Virusateologie der Hepatitis, Arch ges Virusforsch *4*, 89-117

Cameron, J D S, 1943, Infective hepatitis, Quart J Med *12*, 139-155

Capps, R B, Bennett, A M, and Stokes, J, Jr, 1950, A prolonged outbreak of infectious hepatitis in nurses due to a group of small children serving as a reservoir of the virus, J Clin Invest *29*, 802-803

Chalmers, T C, et al., 1955, The treatment of acute infectious hepatitis Controlled studies of the effects of diet, rest, and physical reconditioning on the acute course of the disease and on the incidence of relapses and residual abnormalities J Clin Invest *34*, 1163-1235

Cockburn, W C, Harrington, J A, Zeitlin, R A, Morris, D, and Camps, F E, 1951, Homologous serum hepatitis and measles prophylaxis, Brit M J *2*, 6-12

Colbert, J W, Jr, 1949, Review of animal experimentation in infectious hepatitis and in serum hepatitis, Yale J Biol & Med *21*, 335-343

Colbert, J W, Jr, Holland, J F, Heissler, I, and Knowlton, M, 1951, The use of ACTH in acute viral hepatitis, New England J Med *245*, 172-177

Drake, M E, Barondess, J A, Bashe, W J, Jr, Henle, G, Henle, W, Stokes, J, Jr, and Pennell, R B, 1953, Failure of convalescent gamma globulin to protect against homologous serum hepatitis, J A M A *152*, 690-693

Drake, M E, Hampil, B, Pennell, R B, Spizizen, J, Henle, W, and Stokes, J, Jr, 1952, Effect of nitrogen mustard on virus of serum hepatitis in whole blood, Proc Soc Exper Biol & Med *80*, 310-313

Drake, M E, Kitts, A W, Blanchard, M C, Farquhar, J D, Stokes, J, Jr, and Henle, W, 1950, Studies on the agent of infectious hepatitis II The disease produced in human volunteers by the agent cultivated in tissue culture or embryonated hen's eggs J Exper Med *92*, 283-297

Dresel, E G, Meding, G, and Weineck, E, 1943, Über das virus der Hepatitis epidemica, Ztschr Immunitatsforsch *103*, 129-136

Droller, H, 1945, An outbreak of hepatitis in a diabetic clinic, Brit M J *1*, 623-625

Ducci, H, and Katz, R, 1952, Cortisone, ACTH and antibiotics in fulminant hepatitis, Gastroenterology, *21*, 357-374

———, 1955, Treatment of acute hepatitis with cortisone and antibiotics, Gastroenterology *29*, 381-390

Duncan, G G, Christian, H A, Stokes, J, Jr, Rexer, W. F, Nicholson, J T, and Edgar, A, 1947, An evaluation of immune serum globulin as a prophylactic agent against homologous serum hepatitis, Am J M Sc *213*, 53-57.

Nelson, J B., 1952, Acute hepatitis associated with mouse leukemia. I. Pathological features and transmission of the disease J Exper Med. *96*, 293-302

Nelson, R. S., 1957, The present indications for cortisone therapy in acute viral hepatitis, Ann Int Med *46*, 685-695

New York Academy of Medicine, 1957, Prevention of viral hepatitis. A report by the Committee on Public Health of the New York Academy of Medicine Bull. New York Acad Med. *33*, 128-131

Niven, J S F., Gledhill, A W., Dick, G W A., and Andrewes, C H., 1952, Further light on mouse hepatitis, Lancet *2*, 1061

Oliphant, J W., Gilliam, A. G., and Larson, C L., 1943, Jaundice following administration of human serum, Pub Health Rep *58*, 1233-1242

Olitsky, P K., and Casals, J., 1945, Certain affections of the liver that arise spontaneously in so-called normal stock albino mice, Proc. Soc Exper. Biol & Med *60*, 48-51

Paine, R S., and Janeway, C A, 1952, Human albumin infusions and homologous serum jaundice, J.A.M.A. *150*, 199-202

Paul, J R., and Gardner, H T, 1950, Endemiologic aspects of hepatitis in U S. troops in Germany, 1946-1950, Am J Med. *8*, 565-580

Paul, J R., Havens, W. P., Jr., Sabin, A B., and Philip, C B, 1945, Transmission experiments in serum jaundice and infectious hepatitis, J.A.M.A *128*, 911-915

Post, J., Gellis, S., and Lindenauer, H J., 1950, Studies on the sequelae of acute infectious hepatitis, Ann Int Med. *33*, 1378-1397.

Read, M R., Bancroft, H., Doull, J A., and Parker, R F., 1946, Infectious hepatitis—presumedly foodborne outbreak, Am J Pub Health *36*, 367-370.

Roholm, K., and Iversen, P., 1939 Leberveranderungen bei akuter epidemischer Hepatitis, Verhandl deutsch Gesellsch inn Med *51*, 359-361

Rubarth, S, 1945, Bidrag till den patolog-anatomiska bilden och etiologin vid den s.k. toxiska lever-dystrofin hos hund. Skandinavisk Veterinartidskrift, pp 356-361

Sawyer, W. A., Meyer, K. F., Eaton, M D., Bauer, J H., Putnam, P., and Schwentker, F. F, 1944, Jaundice in Army personnel in the western region of the United States and its relation to vaccination against yellow fever Part I Am J Hyg *39*, 337-430, Parts II, III and IV Am J Hyg *40*, 35-107

Sborov, V M, Giges, B., and Mann, J D, 1953, Incidence of hepatitis following use of pooled plasma A follow-up study in five hundred eighty-seven Korean casualties A.M.A Arch Int Med. *92*, 678 683.

Shaffer, J M, Farquhar, J D, Stokes, J, Jr, and Sborov, V M, 1950, Studies on the use of aureomycin in hepatic disease I Aureomycin therapy in acute viral hepatitis Am J. M Sc *220*, 1-5.

Sherlock, S, 1948, Post-hepatitis cirrhosis, Lancet *1*, 817-822

Sherlock, S., and Walshe, V., 1946, The post-hepatitis syndrome, Lancet *2*, 482-484

Sherman, I L., and Eichenwald, H F., 1956, Viral hepatitis descriptive epidemiology based on morbidity and mortality statistics, Ann Int. Med *44*, 1049-1069

Siede, W., and Meding G., 1941, Zur Ätiologie der Hepatitis epidemica, Klin. Wchnschr. *20*, 1065-1067

Stokes, J., Jr, Farquhar, J A, Drake, M E., Capps, R B., Ward, C S, Jr., and Kitts, A W., 1951, Infectious hepatitis Length of protection by immune serum globulin (gamma globulin) during epidemics, J.A.M.A *147*, 714-719

Stokes, J., Jr., and Neefe, J R., 1945, The prevention and attenuation of infectious hepatitis by gamma globulin, J.A.M.A *127*, 144-145

Stokes, J., Jr., et al, 1954, The carrier state in viral hepatitis, J.A.M.A *154*, 1059-1065

Swift, W E., Jr., Gardner, H T., Moore, H J., Streitfeld, F H., and Havens, W P., Jr, 1950a, Clinical course of viral hepatitis and the effect of exercise during convalescence, Am J Med. *8*, 614-622

Swift, W E., Jr, Miller, W N, Streitfeld, F H, and Knowlton, M., 1950b, An evaluation of two screening tests for the detection of early and subicteric viral hepatitis, Am J Med. *8*, 581-583

Symposium on the Laboratory Propagation and Detection of the Agent of Hepatitis, 1954 Publication *322*, pp 1-115, National Academy of Sciences —National Research Council, Washington

Tucker, C B, Owen, W H, and Farrell, R P, 1954, An outbreak of infectious hepatitis apparently transmitted through water, South M J *47*, 732-739

Turner, R. H., Snavely, J R, Grossman, E B., Buchanan, R. N., and Foster, S O, 1944, Some clinical studies of acute hepatitis occurring in soldiers after inoculation with yellow fever vaccine with especial consideration of severe attacks, Ann Int Med. *20*, 193-218

Voegt, H., 1942, Zur Aetiologie der Hepatitis epidemica, München med Wchnschr *89*, 76-79

Ward, R, and Krugman, S., 1957, Personal communication

Ward, R, Krugman, S, Giles, J, and Jacobs, M A, 1957, Endemic viral hepatitis in an institution epidemiology and control *in* Hepatitis Frontiers, p 227, edited by Hartman, F W., et al, Boston, Little

Watson, C J, and Hoffbauer, F W., 1946, The problem of prolonged hepatitis with particular reference to the cholangiolitic type and to the development of cholangiolitic cirrhosis of the liver, Ann Int Med *25*, 195-227

Wewalka, F., 1953, Zur Epidemiologie des Ikterus bei der antisyphilitischen Behandlung, Schweiz Ztschr allg Path *16*, 307-312

World Health Organization Technical Report Series No 62, 1953, Expert Committee on Hepatitis First Report

J, 1947, Infectious hepatitis in childhood, J Pediat *30*, 381-387

Janeway, C A, 1945, Use of concentrated human serum γ-globulin in the prevention and attenuation of measles, Bull New York Acad Med *21*, 202-222

Jersild, M, 1945, Stigende hyppighed af hepatitis chronica, Ugesk laeger *107*, 819-826

Jordan, J, and Mirick, G S, 1955, An infectious hepatitis of undetermined origin in mice I Description of the disease J Exper Med *102*, 601-616

Klatskin, G, and Rappaport, [redacted] M, 1947, Late residuals in presumably cured acute infectious hepatitis, Ann Int Med *26*, 13-26

Kunkel, H G, and Labby, D H, 1950, Chronic liver disease following infectious hepatitis II Cirrhosis of the liver Ann Int Med *32*, 433-450

Kunkel, H G, Labby, D H, and Hoagland, C L, 1947, Chronic liver disease following infectious hepatitis I Abnormal convalescence from initial attack Ann Int Med *27*, 209-219

Lackey, M D, Eichman, P L, and Havens, W P, Jr, 1953, Hepatitis in laboratory mice, J Infect Dis *93*, 14-20

Lucke, B, and Mallory, T, 1946, The fulminant form of epidemic hepatitis, Am J Path *22*, 867-945

Lucké, B, and Radcliffe, H, 1949, Virus hepatitis of birds and its possible relation to epidemic hepatitis of man, Tr A Am Physicians *62*, 83-89

MacCallum, F O, 1953, Hepatitis, Brit M Bull *9*, 221-226

MacCallum, F O, and Bauer, D J, 1944, Homologous serum jaundice, transmission experiments with human volunteers, Lancet *1*, 622-627

MacCallum, F O, and Bradley, W H, 1944, Transmission of infective hepatitis to human volunteers, Lancet *2*, 228

MacCallum, F. O, McFarlan, A M, Miles, J A R, Pollock, M R, and Wilson, C, 1951, Infective Hepatitis Med Res Council Spec Rep Series, No *273*, pp *103-144*

MacCallum, F O, and Miles, J A R, 1946, A transmissible disease in rats inoculated with material from cases of infective hepatitis, Lancet *1*, 3-4

McCollum, R W, 1952, Size of serum hepatitis virus Proc Soc Exper Biol & Med *81*, 157-160

McNalty, A S, 1938, Acute infectious jaundice and administration of measles serum Annual report of the chief medical officer of the Ministry of Health for the year 1937 London, Her Majesty's Stat Off

Melnick, J L, 1957, A water-borne urban epidemic of hepatitis *in* Hepatitis Frontiers, [redacted] 211, edited by Hartman, F W, et al, Boston, Little

Mirick, G S, 1957, Personal communication

Muller, T, 1947, Hepatitis epidemica mit hoher Letalitat im Kanton Basel-Stadt im Jahre 1946, Schweiz med Wchnschr *77*, 796-802

Murphy, W J, Petrie, L M, and Work, S D, Jr, 1946, Outbreak of infectious hepatitis, apparently milk-borne, Am J Pub Health *36*, 169-173

Murphy, W P, Jr, and Workman, W. G, 1953, Serum hepatitis from pooled irradiated dried plasma, J A M A *152*, 1421-1423

Murray, R, 1954, Source, potency, and availability of clinical material for laboratory testing *in* Symposium on the Laboratory [redacted]ropagation and Detection of the Agent of Hepatitis, Publication 322, pp 101-105, National Academy of Sciences—National Research Council, Washington

———, 1955, Viral hepatitis, Bull New York Acad Med *31*, 341-358

Murray, R, and Diefenbach, W C L, 1953, Effect

M

and blood products, New York J Med 53, 1145-1150

Murray, R, Diefenbach, W C L, Ratner, F, Leone, N C, and Oliphant, J W, 1954a, Carriers of hepatitis virus in the blood and viral hepatitis in whole blood recipients II Confirmation of carrier state by transmission experiments in volunteers J A M A *154*, 1072-1074

Murray, R, Oliphant, J. W, Tripp, J T, Hampil, B, Ratner, F, Diefenbach, W C L, and Geller, H, 1955b, Effect of ultraviolet radiation on the infectivity of icterogenic plasma, J A M A *157*, 8-14

Murray, R, Ratner, F, Diefenbach, W C L, and Geller, H., 1954b, Effect of storage at room temperature on infectivity of icterogenic plasma, J A M A *155*, 13-15

Neefe, J R, Gellis, S S, and Stokes, J, Jr, 1946, Homologous serum hepatitis and infectious (epidemic) hepatitis studies in volunteers bearing on immunologic and other characteristics of the etiological agents Am J Med *1*, 3-22

Neefe, J R, Norris, R F, Reinhold, J G, Mitchell, C B, and Howell, D S, 1954, Carriers of hepatitis virus in the blood and viral hepatitis in whole blood recipients I Studies on donors suspected as carriers of hepatitis virus and as sources of post-transfusion viral hepatitis J A M A *154*, 1066-1074

Neefe, J R, and Stokes, J, Jr, 1945, An epidemic of infectious hepatitis apparently due to a water borne agent J A M A *128*, 1063-1075

Neefe, J R, Stokes, J, Jr, Baty, J [redacted], and Reinhold, J G, 1945a, Disinfection of water containing causative agent of infectious (epidemic) hepatitis, J A M A *128*, 1076-1080

Neefe, J R, Stokes, J, Jr, and Gellis, S S, 1945b, Homologous serum hepatitis and infectious (epidemic) hepatitis Experimental study of immunity and cross immunity in volunteers A preliminary report Am J M Sc *210*, 561-575

Neefe, J R, Stokes, J, Jr, and Reinhold, J G, 1945c, Oral administration to volunteers of feces from patients with homologous serum hepatitis and infectious (epidemic) hepatitis, Am J M Sc *210*, 29-32

Neefe, J R, Stokes, J, Jr, Reinhold, J G., and Lukens, F. [redacted] W, 1944, Hepatitis due to the injection of homologous blood products in human volunteers, J. Clin Invest. *23*, 836-855.

man to man (Andrewes et al, 1951, Lovelock et al, 1952)

CLINICAL PICTURE

The incubation period appears to be relatively short. In transmission experiments in man, it was generally 2 or 3 days in duration with a range of 1 to 6 days (Andrewes, 1949) The onset is often fairly abrupt, and a feeling of roughness, soreness or dryness of the pharynx may be the first symptom Commonly, there is a sensation of irritation, fullness or congestion in the upper respiratory tract, particularly in the nasal passages or the nasopharynx Attacks of sneezing occur early and frequently A thin and watery nasal discharge, which may be copious, is almost invariably present in the early stages The discharge may become viscous or mucoid but rarely does it become mucopurulent before the second or third day An irritating nonproductive cough is present in about 30 per cent of patients Headache, malaise, chilly sensations, some aching of the extremities and lassitude are common complaints At times there is a slight increase in temperature, but this seldom exceeds 100° to 101° F

The nasal and the nasopharyngeal mucosa may be swollen, boggy and injected, one or both nostrils may become partially or completely occluded Postnasal discharge is uncommon during the first few days but may develop later The conjunctival vessels may be prominent, and the eyes may appear suffused The mucous membrane of the fauces and the posterior pharynx may be mildly injected The voice may become husky The upper anterior cervical lymph nodes may become slightly enlarged or tender The olfactory sense is usually diminished and may be lost temporarily The sense of taste may be reduced, and hearing may be moderately impaired Excoriations often develop at the nasal orifices, especially if the nasal discharge is copious In some persons herpetic lesions commonly appear on one or both lips (cf. Chap 38) The course is variable both as to duration and severity In children, particularly in the very young, colds tend to be more severe Fever is often a more prominent symptom, malaise and lassitude are more marked. The appetite may be markedly diminished

If complications do not develop, symptoms seldom persist longer than a week or two and in some colds may disappear after a few days Secondary bacterial infection of some area of the respiratory tract develops frequently, particularly in young children; the paranasal sinuses, middle ears, tonsils, pharynx, larynx, trachea or bronchi and even the lungs may be invaded Any of the potentially pathogenic micro-organisms present in the upper respiratory tract may lead to secondary infection

PATHOLOGIC PICTURE

The mucous membrane of the upper respiratory tract, especially that of the nose, is swollen and boggy and appears inflamed The lymphoid follicles in the affected area are somewhat enlarged There is a striking increase in secretion, both serous and mucous, from the nose Frequently, after some days purulent exudate appears on the surface of the turbinates and the walls of the nasopharynx In the early stages the nasal secretions contain relatively few cells or bacteria and may even appear to be sterile when cultured, later in the course of the disease considerable numbers of cells and various bacteria may be present The chief alterations are confined to the mucous membrane, vascular engorgement, edema and hypersecretion predominate, infiltration by lymphocytes and mononuclear cells occurs in mild degree, as does desquamation of surface cells; necrosis is usually absent

EXPERIMENTAL INFECTION, HOST RANGE

That bacteria-free filtrates of nasal secretions from patients with natural colds are capable of inciting experimental colds in man and the chimpanzee was demonstrated by Olitsky and McCartney (1923) and by Dochez et al (1930) The elaborate and extended investigation of Andrewes and his co-workers (Andrewes, 1949, 1950, 1953, 1958; Andrewes et al, 1951, Lovelock et al, 1952, Andrewes et al, 1953) with volunteers have demonstrated that small filterable agents present in the nasal washings of

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28

Common Cold

(SYNONYMS Acute coryza, acute rhinitis)

INTRODUCTION

The common cold is widely recognized as a relatively mild and almost ubiquitous clinical syndrome which may be induced by one or more of several etiologic factors A considerable proportion of colds can be attributed to a viral infection of the upper respiratory tract. That there may be a number of viruses, each of which can incite the syndrome, seems probable Closely similar maladies which can be indistinguishable on clinical grounds are known to be induced at times by viruses which are associated with other disease categories such as influenza, acute respiratory disease and primary atypical pneumonia Some colds may not be induced by infectious agents but may arise from allergic or traumatic influences.

The symptoms and signs suggest that the syndrome results from an acute but transient alteration in the physiology of the mucous membrane of the upper respiratory tract, particularly that covering the nasal passages, the turbinates and the paranasal sinuses The syndrome develops in man more frequently than any other ailment and leads to an enormous total morbidity each year The incidence of colds is considered to be approximately 2 to 4 attacks per person per year Of themselves, colds are usually relatively mild disorders which generally do not linger for more than a week or two However, they may lead to secondary bacterial infections, especially of the paranasal sinuses, the middle ear, the larynx, the trachea and the bronchi, which may be protracted and occasionally are serious

HISTORY

The clinical syndrome was described in some of the earliest medical writings, and innumerable papers concerning it have appeared during the past several decades. Kruse, 1914, and Foster, 1916, were the first to report successful experimental transmission of colds with filtered material in man Numerous independent reports have appeared which confirm these early findings Dochez et al, 1930, first reported successful transmission of the common cold from man to chimpanzees by means of filtrates Cultivation in tissue-culture medium by Dochez et al (1931) and on the chorio-allantoic membrane of the chick embryo by Kneeland et al (1936) of a filterable agent capable of inducing colds in man has been described

The extensive investigations carried out with large numbers of human volunteers at the Common Cold Research Unit, Salisbury, England, since 1946 (Andrewes, 1949, 1950, 1953, 1958) have reaffirmed the infective nature of certain colds, the filterability of an inciting agent, and have provided much information on the transmission of colds from

virus or group of viruses. The agent is filterable through Berkefeld W candles and Seitz pads (Dochez et al., 1930). Studies with collodion membranes of graded porosity indicate that the agent may have dimensions of less than 70 mμ and possibly is as small as 30 mμ (Andrewes, 1949, 1953). It is not inactivated by penicillin or other antimicrobial agents but is inactivated in vitro by 20 per cent ethyl ether. It can be preserved and remains infectious for man for 2 years or more at −76° C. Conflicting results have been obtained in attempts to preserve the agent by freezing and drying in vacuo (Dochez et al., 1931, Andrewes, 1953).

As indicated above, the agent has not induced a recognizable infection in any species of laboratory animal tested, excepting only the chimpanzee (Dochez et al., 1930), and has not been propagated in series in any tissue culture system, excepting only cultures of human embryonic lung (Andrewes et al., 1953). In volunteers, after intranasal instillation of nasal washings secured from patients with natural colds, the incidence of experimental colds is about 50 per cent (Andrewes, 1949, 1953, 1958). Women appear to be slightly more susceptible to induced colds than men, the observed incidence in a very large series has been 55 per cent and 43 per cent, respectively (Andrewes, 1953). Neither age, between 18 and 40 years, nor the duration of the interval since the last cold nor the season of the year affected these rates appreciably. The most active nasal washings can be diluted as much as 1:1,000, but not 1:10,000, and will still produce colds in man.

The agent can be recovered from inoculated volunteers about 24 hours after intranasal instillation or approximately the same period before symptoms appear. This suggests that patients may be discharging the agent during the latter part of the incubation period as well as during the full-blown cold (Andrewes, 1950, 1953). How long the agent persists in the nasal secretions after a cold is not yet known, but it has been demonstrated in washings obtained as late as the 7th day. Whether the agent is present at times in the upper respiratory tract of apparently normal persons remains an open question. Because either pooled washings from normal persons or even normal embryonated egg materials have at times lead to the development of a cold on intranasal instillation in volunteers, this possibility has been considered (Andrewes, 1950).

No satisfactory serologic procedure has been devised with this agent and, as a consequence, it has not been feasible to carry out antigenic analyses or to make comparisons among various strains. Tests for immunity on second inoculation, either in man or in the chimpanzee, have not yet proved sufficiently satisfactory or reproducible to permit a study of the immunologic relations between different strains.

Serum from persons convalescent from colds may have the capacity to inactivate the agent, but it has not yet been established that this effect is produced by specific antibodies (Andrewes, 1950). The results of tests for immunity to second infection in chimpanzees suggest that some resistance develops and may persist for 3 or 4 months. The evidence regarding the possible development of resistance to second infection in man is not consistent. Pollard and Caplovitz (1948) reported that volunteers were refractory to infection on reinoculation for at least 13 days. On the other hand, the Commission on Acute Respiratory Diseases (1949) found no evidence of resistance to infection on reinoculation with the same nasal washings 19 to 21 days after the first inoculation. Andrewes (1950, 1953) has concluded that susceptibility to experimental colds in volunteers is not correlated with the time elapsed after the last cold.

Various groups of workers in the United States have reported that agents obtained from nasal secretions or washings of patients with colds could be passed in series in the chick embryo (Kneeland et al., 1936, Pollard and Caplovitz, 1947, Topping and Atlas, 1947, Ward and Proctor, 1950, Reagan et al., 1954, Pollard, 1956). The available data is not sufficient to support any conclusion regarding the relationship of these agents to each other or to the virus described by the British workers. Because of the uncertainties that continue to plague investigators in this field and the need to depend on elaborate and carefully controlled tests in volunteers before concluding that a cold-inducing agent is in

patients with colds can induce colds in about 50 per cent of test subjects when they are inoculated into the nasal passages. These agents have consistently failed to produce evidence of infection in a wide variety of animal species, including several species of primate, and it has not been possible even to demonstrate survival of the agents in their nasal passages (Andrewes, 1953). They could not be cultivated in embryonated eggs—in the yolk sac, the amniotic or the allantoic cavities—but in some instances did appear to survive in cultures of human embryonic nasal epithelium (Andrewes, 1953). One such agent was propagated through 10 serial cultures in human embryonic lung, in which it did not produce cytopathogenic effects, and remained capable of inducing colds in volunteers (Andrewes et al., 1953).

Cultivation of a cold-inducing agent in a tissue-culture medium has been reported by Dochez et al. (1931) and by Powell and Clowes (1931) In addition, an agent with similar capabilities has been cultivated on the chorio-allantoic membrane of the chick embryo by Kneeland et al. (1936). Several groups of workers in the United States have reported that agents from patients with colds could be cultivated in series in the allantoic sac of the chick embryo (Pollard and Caplovitz, 1947; Topping and Atlas, 1947; Ward and Proctor, 1950; Reagan et al, 1954; Pollard, 1956) These results clearly do not correspond to those secured during the same period by the British workers at the Common Cold Research Unit. Unequivocal explanations for the striking differences that have been reported are not yet available.

The induction of a coldlike infection in suckling hamsters has been reported by Reagan et al. (1956) but Jordan and Denny (1957) have reported that they were unable to transmit colds to these animals Reports of transmission to other small laboratory animals have not appeared

Price (1956) has described an agent, recovered from patients with coldlike symptoms, which produced cytopathogenic effects in monkey kidney cells in vitro and was transmissible in them in series. This agent did not cause degeneration of HeLa cells in culture and did not multiply in either the allantoic or the amniotic sac of the chick embryo. It did not produce evidence of infection in 1-day-old mice, 10-day-old hamsters, young guinea pigs, or ferrets.

Pelon et al. (1957) have reported the recovery of a cytopathogenic agents from adults with mild respiratory illnesses, many of which presented the clinical syndrome of the common cold. This agent also was transmissible in series in monkey kidney cells in vitro and caused destruction of epithelial cells. In addition, cytopathogenic effects were found in cultures of monkey testis and human embryo kidney but not in monkey cornea, chick embryo, HeLa, KB or human conjunctival cells This agent did not produce evidence of infection in the yolk, the allantoic or the amniotic sac of the chick embryo or in 1-day-old mice inoculated by several routes

ETIOLOGY

The concept that the clinical syndrome designated the common cold includes one or more specific disease entities induced by a virus is now widely accepted. The early and extended studies of Dochez and his co-workers (Dochez et al, 1930, 1931; Kneeland et al, 1936) provided the strongest support for this hypothesis that had been obtained up to that time. The more recent and even more comprehensive investigations carried out continuously since 1946 by the Common Cold Research Unit in England (Andrewes, 1949, 1950, 1953, 1958, Andrewes et al, 1951, 1953; Lovelock et al, 1952) have provided evidence that appears to be conclusive. Whether there is but one variety of common cold virus or a number of dissimilar viruses each of which can incite the same clinical syndrome, as in the case of influenza (cf. Chap 31), is not yet clear.

One of the major objectives of the British group of investigators was to use newer virus technics in attempts to discover a method of recognizing and titrating the cold virus or viruses with which they worked in the laboratory. This important objective has not yet been attained (Andrewes, 1953, 1958), and in consequence it has continued to be essential to conduct all definitive experiments in volunteers. As a result of carefully controlled tests in man the following facts have been established concerning the properties of this

upper respiratory tract, local irritations of the nose or the nasopharynx and a number of viral diseases in mild form, particularly influenza, acute respiratory disease, primary atypical pneumonia and abortive measles In addition, the rhinitis that is commonly associated with the onset of various other disease entities such as varicella, rubella and pertussis may closely simulate the common cold

TREATMENT

No clearly effective or specific method of treatment has been devised Symptomatic therapy appears not to alter significantly the course of the condition, although it often gives the patient some relief from the more distressing symptoms It has been claimed that various so-called antihistaminic drugs can be used as prophylactic or therapeutic agents Numerous carefully controlled studies, both in naturally occurring and in induced colds have failed entirely to affirm such claims (Feller et al 1950 Hoagland et al, 1950, Cowan and Diehl 1950 Medical Research Council, 1950 Shaw and Wightman 1951)

EPIDEMIOLOGY

The syndrome occurs throughout the world in every climate, and at any time There appear to be seasonal variations in incidence In the temperate zones the attack rate tends to rise during both the fall and the spring seasons In isolated Arctic communities the attack rate decreases markedly during the winter, the greater the degree of isolation, the lower is the incidence (Heinbecker and Irvine-Jones 1928, Paul and Freese, 1933) Re-establishment of contact with other communities often leads to a striking increase in the number of colds Whether this is attributable to the introduction of different strains of the causal agent or to a gradual decline in resistance to infection under conditions of isolation is not yet clear It is thought that the agent or agents are transmitted from one person to another under natural conditions, presumably by air-borne droplets However, attempts to induce contact infections under carefully controlled conditions have been surprisingly unrewarding (Andrewes, 1950, Andrewes et al, 1951, Lovelock et al, 1952). The idea that drafts and chilling tend to be associated with the development of colds seems to be firmly entrenched. Direct tests of the effects of chilling on susceptibility to experimental infection have failed to reveal any clear results (Andrewes, 1950)

Epidemiologic studies by Lidwell and Sommerville (1951) indicated that the probability of contracting a cold was about 1 in 5 when the infection was introduced into a household Adults in households in which there was a school-age child appeared to develop twice as many colds as others who lacked this close contact with children. Extensive investigations on respiratory infections in a group of families by Dingle and his co-workers (Dingle et al, 1949, Badger et al, 1953) indicate that respiratory diseases accounted for about 75 per cent of all episodes of illness and that some 40 per cent of respiratory illnesses were common colds.

CONTROL MEASURES

No effective control measures have been devised Various bacterial vaccines, designed presumably with a view to decreasing susceptibility to secondary bacterial infection, have been widely used, but carefully controlled studies have failed to provide evidence of a significant effect (Ferguson et al 1927, Diehl et al, 1938) Because of the lack of technics for the cultivation of viruses that are clearly associated with colds it has not been feasible to develop vaccines containing such viruses or test their possible efficacy Because of the apparent brief duration of immunity after either natural or experimental colds it has seemed probable that a vaccine of this kind might not be promising (Andrewes, 1953).

REFERENCES

(For complete references, see also 2nd edition, Chapter 16)

Andrewes, C. H., 1949, The natural history of the common cold, Lancet 1, 71-75

———, 1950, Adventures among viruses, III The puzzle of the common cold New England J Med 242, 235-240

———, 1953, The common cold, Brit M Bull 9, 206-207

———, 1955, A decade with the common cold, Bull. Johns Hopkins Hosp 103, 1-7.

fact present, it is not yet possible to resolve the several conflicting results satisfactorily.

In addition to the agents indicated above, several new agents have been described in the past few years These agents all cause cytopathogenic effects in certain tissue culture systems and can be manipulated readily by the newer virus technics Further work will be needed before the extent of the relationship of any of them to the common cold becomes clarified The cytopathogenic agents recently reported by Price (1956) and Pelon et al (1957) appear to possess certain properties in common Both were recovered from patients with mild respiratory illnesses who showed syndromes that appeared to correspond with that of the common cold Both are transmissible in series in monkey kidney cell cultures and produce cytopathogenic effects Neither induces infection in HeLa cell cultures, chick embryo tissues or 1-day-old mice Both agents pass through filters that retain bacteria, and neither is inactivated by antimicrobial substances They are readily neutralized by convalescent sera of patients with homotypic disease, a definite neutralizing antibody response has been demonstrated by comparing the neutralization titers of acute phase and convalescent sera. Neutralizing antibodies against both agents were found with moderate frequency in the sera of children over 8 years of age and young adults Complement-fixing antibodies also were demonstrated with one of the agents (Price, 1946) but not with the other (Pelon et al, 1947) Hemagglutinating activity was demonstrable only with one (Pelon et al, 1947) Both agents were tested with numerous sera containing antibodies against a considerable number of other viruses and some rickettsiae In no instance was evidence obtained indicating an antigenic relationship between agents of established identity and these cytopathogenic agents There are as yet no published reports of cross immunologic tests with the two agents. However, on the basis of preliminary experiments Mogabgab (personal communication) considers that the two agents may be antigenically related, although they do not appear to be identical Apparently neither agent has as yet been tested directly in volunteers by the workers who recovered it to determine the clinical features of the experimental infection it might induce in man The JH virus (Price, 1956) after serial passage in monkey kidney cultures was tested in volunteers in England but appears not to have incited colds (Andrewes, personal communication). Recently, Price (1957) reported that an inactivated vaccine prepared with the JH virus protected children for several weeks against the overt illness due to this virus.

Cytopathogenic agents have been recovered by Sabin (cf. Ramos-Alvarez and Sabin, 1956) and by Morris et al (1956) from chimpanzees during outbreaks of naturally occurring mild respiratory illnesses simulating the common cold The chimpanzee rhinitis virus, which was recovered in 1954, appears to be related to ECHO type 10 virus (Sabin, 1956). The virus causes cytopathogenic effects in monkey kidney cultures and can be transmitted in series On nasal instillation it induced manifest rhinitis in chimpanzees which was associated with the development of neutralizing antibodies Antibodies against it have been demonstrated in man; although infants had none, children and young adults showed a progressively increasing incidence (Sabin, personal communication) The chimpanzee coryza agent (Morris et al, 1956) produced cytopathogenic effects in human liver cell cultures (Chang strain) and was propagated in series It appeared to be different from the rhinitis virus in serologic tests Antisera against a number of other viruses of established identity failed to react with the coryza agent A number of human beings, particularly adolescents and young adults, were found to have antibodies against the agent in their sera

DIAGNOSIS

The diagnosis is dependent entirely upon the clinical findings which in many cases are sufficiently characteristic to suggest the nature of the syndrome There is no laboratory test the results of which will positively support the diagnosis Many conditions may cause clinical syndromes closely similar to, if not identical with, that of the common cold, among these are various forms of nasal allergy, a variety of mild bacterial infections of the

strains of the common cold virus in suckling hamsters, A.M.A. Arch. Path. *62*, 183-184
Sabin, A. B., 1956, Conference on viruses in search of disease, Ann. New York Acad. Sc. *67*, 250-252
Shaw, C. R., and Wightman, H. B., 1951, Further studies on the treatment of the common cold with antihistamine drugs, New York J. Med. *51*, 387-388
Topping, N. H., and Atlas, L. T., 1947, The common cold: a note regarding isolation of an agent, Science *106*, 636-637
Ward, T. G., and Proctor, D. F., 1950, Isolation of a common cold virus in chick embryos and the clinical manifestations it produces in human volunteers, Am. J. Hyg. *52*, 91-106

Andrewes, C H, Chapromere, D M, Compels, A E H, Pereira, H G, and Roden, A T, 1953, Propagation of common-cold virus in tissue cultures, Lancet *2*, 546-547

Andrewes, C H, Lovelock, J E, and Sommerville, T, 1951, An experiment in the transmission of colds, Lancet *1*, 25-27

Badger, G F, Dingle, J H, Feller, A E, Hodges, R G, Jordan, W S, Jr, and Rammelkamp, C H, Jr, 1953, A study of illness in a group of Cleveland families II Incidence of the common respiratory diseases. Am J Hyg *58*, 31-40

Commission on Acute Respiratory Diseases, 1949, Experimental transmission of minor respiratory illness to human volunteers by filter-passing agents II Immunity on reinoculation with agents from the two types of minor respiratory illness and from primary atypical pneumonia J Clin Invest *26*, 974-982

Cowan, D W, and Diehl, H S, 1950, Antihistaminic agents and ascorbic acid in the early treatment of the common cold, J A M A *143*, 421-424

Diehl, H S, Baker, A B, and Cowan, D W, 1938, Cold vaccines An evaluation based on a controlled study J A M A *111*, 1169-1173

Dingle, J H, Badger, G F, Feller, A E, Hodges, R G, Jordan, W S, Jr, and Rammelkamp, C H, Jr, 1949, A study of respiratory infections in families, Tr A Am Physicians *62*, 99-104

Dochez, A R, Mills, K C, and Kneeland, Y, Jr, 1931, Study of the virus of the common cold and its cultivation in tissue medium, Proc Soc Exper Biol & Med *28*, 513-516

Dochez, A R, Shibley, G S, and Mills, K C, 1930, Studies in the common cold IV Experimental transmission of the common cold to anthropoid apes and human beings by means of a filterable agent J Exper Med *52*, 701-716

Feller, A E, Badger, G F, Hodges, R G, Jordan, W S, Jr, Rammelkamp, C H, Jr, and Dingle, J H, 1950, The failure of antihistaminic drugs to prevent or cure the common cold and undifferentiated respiratory diseases, New England J Med *242*, 737-744

Ferguson, F R, Davey, A F C, and Topley, W W. C, 1927, The value of mixed vaccines in the prevention of the common cold, J Hyg *26*, 98-109

Heinbecker, P, and Irvine-Jones, E I M, 1928, Susceptibility of Eskimos to the common cold and a study of their natural immunity to diphtheria, scarlet fever and bacterial filtrates, J Immunol *15*, 395-406

Hoagland, R J, Dietz, E N, Meyers, P W, and Cosand, H C, 1950, Antihistaminic drugs for colds Evaluation based on a controlled study J A M A *143*, 157-160

Jordan, W. S, Jr, and Denny, F W, Jr, 1957, Failure to transmit common cold to suckling hamsters, Proc Soc Exper. Biol & Med *95*, 651-653

Kneeland, Y, Jr, Mills, K. C, and Dochez, A R, 1936, Cultivation of the virus of the common cold in the chorio-allantoic membrane of the chick embryo, Proc Soc Exper. Biol & Med. *35*, 213-215

Lidwell, O. M, and Sommerville, T, 1951, Observations on the incidence and distribution of the common cold in a rural community during 1948 and 1949, J Hyg *49*, 365-381

Lovelock, J. E, Porterfield, J. S, Roden, A T, Sommerville, T, and Andrewes, C H, 1952, Further studies on the natural transmission of the common cold, Lancet *2*, 657-660

Medical Research Council Special Committee, 1950, Clinical trials of antihistaminic drugs in the prevention and treatment of the common cold, Brit M J. *2*, 425-429

Morris, J A, Blount, R E, Jr, and Savage, R E, 1956, Recovery of cytopathogenic agent from chimpanzees with coryza, Proc Soc Exper Biol & Med *92*, 544-549

Olitsky, P K, and McCartney, J. E, 1923, Studies on the nasopharyngeal secretions from patients with common colds, J Exper Med *38*, 427-440

Paul, J H, and Freese, H L, 1933, An epidemiological and bacteriological study of the "common cold" in an isolated Arctic community (Spitsbergen), Am J Hyg *17*, 517-535

Pelon, W, Mogabgab, W J, Phillips, I A, and Pierce, W. E, 1957, A cytopathogenic agent isolated from Naval recruits with mild respiratory illnesses, Proc Soc Exper Biol & Med *94*, 262-267

Pollard, M, 1956, Cytological response in embryonated eggs to inoculums from cases of common cold infection, Proc Soc Exper Biol & Med *91*, 449-453

Pollard, M, and Caplovitz, C D, 1947, Experimental studies with the agent of the common cold, Science *106*, 243-244

———, 1948, Immunological studies with common cold infection, Am J Hyg *47*, 106-112

Pollard, M, Dernehl, C U, and Caplovitz, C D, 1948, Survival of the virus of the common cold in specimens collected from naturally acquired cases of common cold, Am J Hyg *47*, 103-105

Powell, H M, and Clowes, G H A, 1931, Cultivation of the virus of common cold and its inoculation in human subjects, Proc Soc Exper Biol & Med *29*, 332-335

Price, W, 1956, The isolation of a new virus associated with respiratory clinical disease in humans, Proc Nat Acad Sc *42*, 892-896

Price, W H, 1957, Vaccine for the prevention in humans of coldlike symptoms associated with the JH virus, Proc Nat Acad Sc *43*, 790-795

Ramos-Alvarez, M, and Sabin, A B, 1956, Intestinal viral flora of healthy children demonstrable by monkey kidney tissue culture, Am J Pub Health *46*, 295-299

Reagan, R L, Palmer, E D, Delaha, E C, Cook, S R, Brueckner, A L, and Nelson, H E, 1954, Electron microscopic studies of an egg-adapted virus isolated from a patient with a common cold, Texas Rep Biol & Med *12*, 1067-1073

Reagan, R L, Palmer, E, Yancey, F S, Chang, S C, and Brueckner, A L, 1956, Studies of four

garding its viral etiology by demonstrating that pneumonia could be transmitted to volunteers with filtered respiratory tract secretions References pertinent to this historical sketch may be found in the previous editions of this volume

CLINICAL PICTURE

The incubation period varies from 1 to 3 weeks, with an average of 12 to 14 days An exact clinical characterization of the disease is impossible because of lack of a specific diagnostic test, for it is likely that cases of mixed etiology have been included in past descriptions (Reimann, 1938 Dingle and Finland, 1942, Curnen et al, 1945, Jordan et al, 1951). However, since many of the cases were observed during epidemics and developed cold hemagglutinins, the pattern of illness woven from these descriptions probably does not represent too great a distortion of the manifestations of an entity

The onset is usually gradual and insidious The clinical features are those of a mild to moderately severe infection, and the early complaints may be referable to either the upper or the lower respiratory passages, or to both, or may be principally constitutional in nature Respiratory symptoms include throat irritation and cough, the latter being the most frequent and characteristic feature Constitutional symptoms include headache, malaise, feverishness, chilliness, fatigue and anorexia Headache is frequently an outstanding complaint and is particularly distressing when the patient coughs The cough is dry and frequently paroxysmal during the first 3 to 5 days of illness Ultimately, it is productive of sputum which is either mucoid or mucopurulent Blood-streaked sputum occurs in less than 10 per cent of cases A variable percentage of patients experience chest pain, which appears to be directly related to the severity of the cough, is substernal in location and is described as a burning sensation or aching discomfort In contrast to bacterial pneumonia, most patients do not appear very ill early in the disease, a shaking chill is rare, the pulse is slow in relation to the fever, respirations are normal or slightly increased; grossly bloody sputum and pleural pain are uncommon; herpes labialis is sel present

Examination usually reveals few striki abnormal physical signs Early in the cou the patient appears to be mildly or mo ately ill with little more than slight infl mation of the throat Minimal dullness percussion and diminution of breath sou may be present but seldom reflect the ex of infiltration demonstrated by roentg grams Early in the disease, the same is of rales Later, the most characteristic pl ical finding is the presence of fine, subc itant, sticky rales in the absence of sign true consolidation As the disease progres rhonchi or coarse rales may be heard

Roentgenographic examination often sh evidence of pneumonia before physical s are apparent, the disproportion between of infiltration and degree of pulmonary volvement demonstrated roentgenographic being an almost constant feature The c solidation usually appears to be most de at the hilum and is progressively less de near the periphery The borders of the pr monic lesions are irregular, and extens seems to occur in patchy fashion along course of the bronchovascular trunks shadows may appear diffuse, mottled, fea ery, or, in rare instances, dense The lo lobes are involved most frequently, altho any area in the lungs may be affected approximately 50 per cent of patients, pn monia is present in only one lobe Spread the infiltration, with resolution of the ea lesions as later ones appear, is not unc mon Thus, the changes are consistent w the concept that the infiltrative process one of peribronchial, peribronchiolar and terstitial inflammation, associated with fc atelectasis and edema The roentgenograp signs may be transitory for a few days may be progressive, they generally unde slow resolution over a period of 1 to 3 wee Residual nodular densities or prominent br chovascular markings may persist for seve weeks longer It is emphasized that the x-picture is not distinctive or diagnostic a is simulated by other forms of pulmon disease

Figure 103 illustrates the course of a c of average severity In this patient, con tutional symptoms, headache and cough w

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29

Primary Atypical Pneumonia

(SYNONYMS Atypical pneumonia, virus pneumonia, acute interstitial pneumonitis)

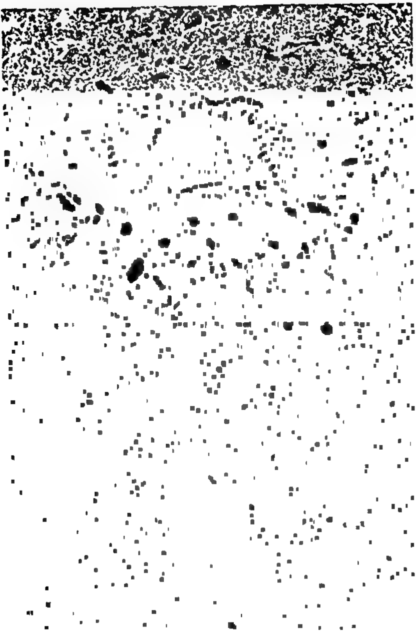
INTRODUCTION

Primary atypical pneumonia is an acute, self-limited respiratory disease characterized by constitutional symptoms, cough, and pulmonary infiltration most readily demonstrated by roentgenogram Obviously, these characteristics are not specific, and similar clinical features may be manifested by certain viral and rickettsial diseases of established etiology, such as psittacosis and Q fever. Therefore, it is possible that more than one unknown agent may be responsible for infections classified as primary atypical pneumonia, accordingly, it has been customary to refer to the disease as a syndrome rather than as a specific entity Justification of this concept was obtained recently with the demonstration that pulmonary infiltration consistent with a clinical diagnosis of primary atypical pneumonia may occur in certain adenovirus infections (Chap 30) In such instances, however, pneumonia due to an adenovirus should be so diagnosed, rather than primary atypical pneumonia, just as a specific diagnosis of influenza is made when primary pneumonitis is found in that disease With the exclusion of known diseases which may present similar clinical syndromes, there emerges a form of pneumonia, often associated with the development of cold hemagglutinins, which appears to be an entity—primary atypical pneumonia.

HISTORY

The differentiation of primary atypical pneumonia from the bacterial pneumonias was accomplished in the late 1930's and early 1940's, but it is probable that some of the similar cases reported previously represented the same disease. Sections of lungs removed from soldiers during the Civil War

I also were instances of primary atypical pneumonia. Between 1930 and 1937, Arrasmith, Gallagher, Bowen, Allen, and Scadding reported cases in adolescents and young adults of pneumonitis characterized by a benign course and a lack of correlation between roentgenographic changes and physical signs In 1938 and 1939, Reimann, Smiley and others described similar cases and classified the disease as a new entity. Subsequently, with differentiation aided by the effectiveness of the sulfonamide drugs and penicillin in the bacterial pneumonias and the occasional epidemic occurrence of this nonbacterial disease, the clinical and epidemiologic features of primary atypical pneumonia were detailed by Kneeland and Smetana, Longcope, Dingle and Finland, and many others. During World War II, the disease occurred commonly, especially among military personnel, and the Commission on Acute Respiratory Diseases provided evidence re-



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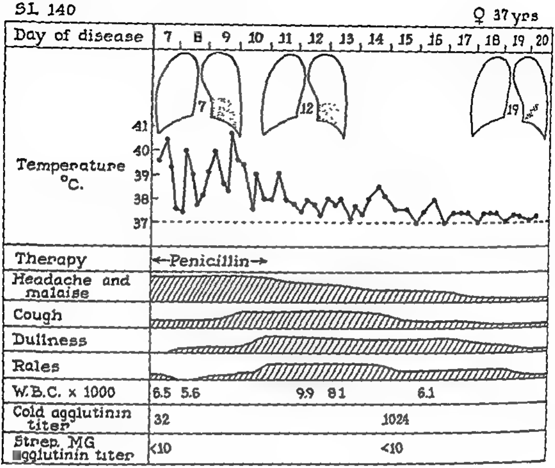

FIG 103. Chart of clinical findings in a case of primary atypical pneumonia

prominent, and at the time of hospitalization on the 7th day, there were no signs of consolidation and few rales despite demonstrable infiltration. Eventually, as happens in most instances, this discrepancy tended to disappear, and the extent of the physical signs became more comparable with the roentgenographic changes. As in the case illustrated, the majority of patients have normal total and differential leukocyte counts. The count may become elevated as the illness progresses, most often in association with spread of the infiltration in the absence of superimposed bacterial complications Erythrocyte counts and hemoglobin values are normal in uncomplicated cases. The sedimentation rate is elevated The urine is normal Culture of the sputum shows bacterial species normally found in the respiratory tract As noted subsequently, a variety of immunologic reactions occur with sera of convalescent patients, the most useful of which is the development of cold hemagglutinins. Occasionally, biologic false-positive Wassermann or Kahn reactions may be obtained.

The course of illness is extremely variable; the duration of fever and the degree to which the temperature is elevated range widely. Fever may be present for from 1 day to 6 weeks, it may be sustained but is most often remittent; it usually terminates by lysis in from 7 to 14 days. During the illness, headache, cough and sweating are commonly the most distressing symptoms. With extensive pulmonary infiltration, dyspnea, cyanosis and abdominal distension occur. In severely ill patients, the infection may aggravate or precipitate cardiovascular dysfunction, including auricular fibrillation, decompensation or circulatory collapse However, such an event is rare; accordingly, the case fatality rate is low (0 1 to 0.2%) (Dingle et al, 1944).

those of the naturally occurring disease and varied from mild respiratory infections to severe pneumonia Of 16 volunteers in whom pneumonia was induced, 13 developed 4-fold or greater increases in titers of cold hemagglutinins in their convalescent sera, and 2 also developed agglutinins against streptococcus MG. Of the 26 patients developing minor respiratory illnesses, without evidence of pneumonia, 11 developed cold hemagglutinins, and in 9 instances the increase was 4-fold The presence or the absence of streptococcus MG in the throat before and after inoculation had no relation to the development of pneumonia nor were there significant changes in the bacterial flora of the respiratory tract before, during or after the illnesses It is apparent that primary atypical pneumonia can be induced in volunteers by a filter-passing agent, presumably a virus, with the development of cold hemagglutinins and, in some instances, of agglutinins for streptococcus MG The results further suggest that the same agent may induce minor respiratory illness without pneumonic involvement

Many attempts have been made in a number of laboratories to transmit primary atypical pneumonia to animals and thus to define the host range of the causative agent or agents Sputa and throat washings obtained early in the course of the disease have been most commonly employed, in a few instances, pulmonary tissue from fatal cases has been used The commonest route of inoculation has been intranasal with the animals under light ether anesthesia Chick embryos have been employed, but thus far there are no reports of attempts using tissue culture The animal species used include the following mice, cotton rats, hooded rats, white rats, hamsters, guinea pigs, rabbits, 2 species of mongooses, 3 species of monkeys, baby chicks, ferrets, rice birds, doves, puppies, dogs, kittens, cats, young pigs and chimpanzees In a great majority of instances no evidence of infection has been found in the animals inoculated Several investigators have induced pulmonary lesions in animals by intranasal inoculation of sputum but have been unable to maintain the lesions on passage to additional animals or have found the same lesions in animals inoculated with control materials

Stokes et al (1939) reported transmission of the disease to mice, guinea pigs and ferrets, using material from 2 patients, but the infection could not be maintained in these animals on passage in the laboratory Weir and Horsfall (1940) reported transmission of the disease to the wild Jamaican mongoose, a finding which was not confirmed by the Commission on Acute Respiratory Diseases in collaboration with Dammin and Weller (1945) using the wild Puerto Rican mongoose Blake, Howard and Tatlock (1942) isolated a transmissible pneumotropic agent from cats ill in a household concomitantly with human cases of primary atypical pneumonia The agent could be transmitted to kittens, cats and hamsters Neutralization tests suggested that the feline and the human illnesses might have been due to the same agent However, later studies did not demonstrate a relationship between the feline agent and other human cases Other infections transmitted to animals have subsequently been found to be due to one of the psittacosis-lymphogranuloma group of viruses, herpes virus, or agents apparently latent in the animals employed (Eaton, 1950)

Eaton et al (1944) reported the production of pneumonic lesions in cotton rats and hamsters following primary intranasal inoculation of sputa and lung On serial passage, the lesions either disappeared or apparently were replaced by lesions due to contaminating or latent viruses The inoculation of chick embryos amniotically with filtered sputum failed to produce any evidence of infection, but lesions could be induced in cotton rats and hamsters inoculated intranasally with 20 per cent suspensions of chick embryo tissue The agent responsible could be maintained on repeated passage in chick embryos More recently, Liu (1957) has reported the transmission of infection to the chick embryo, using sputa and lungs from cases, as indicated in two ways by the fluorescent antibody technic and by the induction of pulmonary consolidation in cotton rats following intranasal inoculation with suspensions of chick embryo tissue Similar findings were obtained by Liu with the strain of virus isolated by Eaton in 1944 Mice and white rats are apparently not susceptible to infection, evidence regarding the guinea pig is indefinite

Resolution begins with return of the temperature to normal, and convalescence is usually uneventful except for asthenia.

Complications are uncommon, but involvement of nearly all organ systems has been reported. sinusitis, dermatitis, pericarditis, myocarditis, encephalitis, polyneuritis and thrombophlebitis Ulcerative tracheobronchitis may persist, but a residual of true bronchiectasis is apparently rare, although follow-up studies have not been carried out extensively enough to permit a reliable appraisal of residual complications Acute hemolytic reactions may occur in certain patients with high titers of cold hemagglutinins, an event usually induced by chilling. Secondary bacterial infection is very uncommon

PATHOLOGIC PICTURE

Relatively few patients in whom the diagnosis was well established have come to au-

al, 1947). Grossly, the lungs are heavier than normal, and areas of congestion and hemorrhage are apparent The pleural surfaces are usually normal but may show patches of fibrinous exudate Occasionally, small amounts of straw-colored pleural fluid are present The pneumonic areas may be extensive and widespread, or discrete, circumscribed and multiple Nodular focal lesions, resembling miliary granulomata, may be present. Various stages of consolidation, as well as areas of atelectasis and emphysema, are seen There is bronchitis and bronchiolitis, and the areas of infiltration are particularly prominent surrounding bronchi and bronchioles. The lumina of these structures contain tenacious exudate The bronchial mucosa is inflamed and ulcerated

Microscopically, the picture is one of interstitial pneumonia and necrotizing bronchitis and bronchiolitis (Fig. 104). The walls of the bronchi and the bronchioles show infiltration with mononuclear and polymorphonuclear cells which may extend into the peribronchiolar tissue and alveolar walls There is necrosis of epithelial cells with desquamation of the mucosa and ulceration The lumina contain this debris mixed with a purulent or mucoid exudate of mononuclear cells, neutrophils and fibrin

The alveoli show thickening of their walls, dilatation of the septal capillaries, edema in varying degrees, and infiltration of the septa by lymphocytes, mononuclear cells and erythrocytes The alveolar spaces may be air-containing, collapsed, or partially filled with exudate and edema fluid Bacteria usually are seen in the pneumonic areas The presence of intracellular inclusion bodies, elementary bodies, or rickettsiae has not been demonstrated

Pathologic alterations in other organs have been observed, particularly acute follicular splenitis, mesenteric lymphadenitis, focal hepatic necrosis, acute myocarditis and hemorrhagic encephalitis

EXPERIMENTAL INFECTIONS, HOST RANGE

The complex etiologic picture of primary atypical pneumonia, as indicated in the next section, renders difficult an orderly discussion of experimental infection and host range. There is no reason to doubt that man himself is susceptible, as indicated by the spontaneous occurrence of the disease and by its experimental transmission to volunteers However, the factor or factors responsible for induction of the spontaneous disease are not known It has been postulated that latent agents may be responsible, activated by some undetermined insult, such as a minor respiratory infection Such latent agents, as for example pneumonia virus of mice (PVM), have been found in a number of animal species and will produce pneumonia in the host animals after nonspecific provocation or after blind passage of suspensions of lung tissue There is no evidence either for or against the presence of similar latent viruses in the lungs of man.

The studies of the Commission on Acute Respiratory Diseases (1946) during World War II indicate that the disease can be transmitted and passed in volunteers under conditions suggesting a viral etiology. The volunteers inhaled a spray of sputa and throat washings obtained from patients and rendered bacteria-free by filtration Approximately 75 per cent of the recipients developed respiratory infections, and 25 per cent of them developed pneumonia, the incubation period being about 12 days. The illnesses resembled

section and described more fully in previous editions. The results of neutralization tests in animals with sera from a limited number of patients suggested that neutralizing antibodies had developed during convalescence. Perhaps the most significant of this latter group of agents is that isolated originally by Eaton et al. (1944) and more recently by Liu (1957). Eaton isolated the agent from approximately 50 per cent of the cases studied and demonstrated 4-fold or greater increases in neutralizing antibody titers in the convalescent sera of approximately 62 per cent of the total examined. Such increases were found most frequently in patients who also showed significant rises in cold hemagglutinins and in agglutinins for streptococcus MG. Using an ingenious, indirect fluorescent antibody technic, Liu studied the behavior of a strain of virus isolated by Eaton in 1944 and that of 8 strains isolated in Boston from sputum or nasal and throat washings, and of another strain isolated from the lung of a patient who died in 1943. Isolations could not be made with material from 11 additional cases. In most of these patients, there was an increase in the titer of cold hemagglutinins and of agglutinins for streptococcus MG in the convalescent sera. The technic consisted of sectioning the pulmonary tissue of chick embryos 5 to 6 days after amniotic inoculation, exposing the sections to antiviral, hyperimmune rabbit serum, or convalescent human serum, and tagging the antibody attached to the virus in the cells with the appropriate fluorescein-labeled antigamma globulin rabbit or human serum. The direct method of staining with fluorescein-labeled antiviral antiserum was not satisfactory because of the low intensity of specific fluorescence. By the use of the indirect technic, the virus was found only in the lower trachea, the bifurcation and the larger branches of the bronchi, and the air sac of infected chick embryos. No histopathologic changes were visible. No virus could be detected in the lungs of cotton rats showing pneumonia after intranasal inoculation of infected chick embryo material. All of the strains of virus isolated were antigenically similar. By this technic, a rise in titer of antibodies was demonstrated in the convalescent sera of 4 of the 8 patients from whom virus was isolated in Boston. Of the other 4 patients, 1, from whom no acute phase serum had been obtained, showed a titer of 1:320 in his convalescent sera, 1 had a titer of 1:160 in both the acute and the convalescent phase serum specimens, and from 2 no sera were available. No cross-reactions could be detected by this method with sera from patients with psittacosis, Q fever, adenovirus infections, or influenza A and B, or in anti-PVM rabbit serum. Liu concluded that the failure of other investigators to isolate Eaton's virus probably is due largely to the low pathogenicity of the virus and its lability. No data have yet been presented with respect to the ability of convalescent sera from cases of primary atypical pneumonia to neutralize Eaton's virus directly in embryonated eggs. Recently, Arakawa et al. (1956) have reported the isolation of a virus from the serum and the pleural fluid of patients with primary atypical pneumonia by intracerebral inoculation of mice and chorioallantoic inoculation of embryonated eggs. The agent apparently produces pneumonia in monkeys and is neutralized by the convalescent sera of patients.

The etiology of primary atypical pneumonia has not yet been clearly defined, and the picture remains confused. In all probability, more than one agent is involved. Final identification of the agent or agents must rest on isolation of the virus from clinically characteristic cases, demonstration of the appearance of antibodies to the agent in convalescent sera, neutralization of viral infectivity by antibodies in convalescent sera and in the sera of animals immunized with the isolated agent, correlation of the antibody level with resistance to infection and, ultimately, by experimental induction of the disease in volunteers or its equivalent. These criteria have not yet been fully met with any agent thus far reported as presumably responsible for the disease. In a general sense, cases resembling primary atypical pneumonia can be divided into 2 groups: (1) those that show the development of cold hemagglutinins or other peculiar serologic reactions, and (2) those that do not exhibit these phenomena. Some, but not all, of the latter group can be shown to be related to infections with psittacosis virus, the adenoviruses, or other agents. The former group may show not only

No other data are thus far available regarding the host range of the Eaton virus.

ETIOLOGY

As already indicated, the clinical features of primary atypical pneumonia are those of a syndrome that may be induced by a variety of infectious and noninfectious agents. Therefore, studies on etiology require a thorough clinical and laboratory investigation of the patient from whom specimens are obtained in order that the known causes of the syndrome can be excluded

Following the discovery of streptococcus MG, Thomas et al (1945) suggested the possibility that this organism might play a causative role. It was isolated from the lungs and the sputa of a number of cases in which no other etiologic agent was apparent. The various strains were serologically homogeneous The bacterium was agglutinated by the convalescent sera of from 35 to 50 per cent of cases of the disease. Thus the isolation of the organism and the development of antibodies in the sera of many patients during convalescence suggested that this streptococcus might be implicated etiologically—alone, or as a secondary invader, or in symbiosis with a virus The present evidence is insufficient to support this concept. The organism is not pathogenic and can be found in approximately 25 per cent of normal persons or patients with other respiratory infections. Pathologically, bacteria appear to have no appreciable part in the production of the lesions of primary atypical pneumonia The presence of streptococcus MG was not related to the experimental transmission of pneumonia in man. It seems most probable that the development of antibodies during convalescence is part of a nonspecific reaction similar to other peculiar nonspecific changes that occur in the sera of these patients during convalescence, or that streptococcus MG shares a common antigen with the causative agent of primary atypical pneumonia as in the case with certain strains of proteus and rickettsia (Weil-Felix reaction)

The results of the transmission studies of the Commission on Acute Respiratory Diseases (1946) summarized in the preceding section, indicate that the disease was at least initiated, if not caused, by a filter-passing agent, presumably a virus, and that the disease so transmitted was associated with the development of cold hemagglutinins In a subsequent study in volunteers (Commission on Acute Respiratory Diseases, 1947), it was found that there was no apparent relationship between primary atypical pneumonia, the common cold and acute respiratory disease (ARD) of recruits, as evidenced by differing clinical pictures, incubation periods, and lack of cross-immunity among the 3 diseases. With the discovery of the adenoviruses, it has been possible to re-evaluate these studies by examining the stored sera from donors and recipients for antibodies to the adenoviruses (Ginsberg et al, 1955). The results showed that the adenoviruses had not been related to these cases of primary atypical pneumonia or to the common cold, but that adenovirus type 4 had been responsible for the cases of acute respiratory disease (ARD).

The results of many attempts to isolate an infectious agent in laboratory animals and to demonstrate its etiologic relationship to the human disease have been conflicting and difficult to interpret. Some of these attempts with materials from both endemic and epidemic cases have been entirely unsuccessful. Adams et al. (1942, 1945) failed to isolate a virus in his studies of 3 epidemics of pneumonitis in infants. However, these outbreaks may have been caused by a different agent, as suggested by the presence of inclusion bodies found at autopsy. Similar inclusion bodies have not been demonstrated in the lungs of adults Other studies have resulted in the isolation of known viruses, such as psittacosis and herpes, or of viruses which clearly were not related to the human disease or were derived from the animals employed. In addition, a virus has been isolated which was termed the newborn pneumonitis virus, type Sendai (Kuroya and Ishida, 1953) and later was classified with the influenza group and designated influenza virus D (Jensen et al, 1955).

Still other investigations have yielded agents that produced what appeared to be infectious processes or lesions in the chick embryo, the hamster, the cotton rat, or the mongoose, as mentioned in the preceding

of cases of psittacosis, Q fever, adenovirus infections and influenza

The significance of the various immunologic reactions that may be obtained with convalescent sera is not clear. Cold hemagglutinins and agglutinins for streptococcus MG appear to be different antibodies, since each can be removed separately by adsorption of a serum containing both of them without appreciably affecting the titer of the other In addition, each may appear independently Both of them are distinct from the antibody for Eaton's virus Other unusual immunologic reactions may also be demonstrated with convalescent sera, such as false-positive serologic tests for syphilis, fixation of complement with fresh tissue antigen of various sorts, and the prevention of development of antibodies to pneumonia virus of mice (PVM) It is entirely possible that all of these are nonspecific reactions dependent in some fashion on the breakdown of pulmonary tissue or on the pulmonary infection

The differential diagnosis requires consideration of a number of infectious and noninfectious diseases that may present a similar or even identical clinical picture Among the bacterial diseases, tuberculosis, tularemia and typhoid should be considered Most of the cases of bacterial pneumonia can be distinguished readily on clinical grounds, but about 5 to 10 per cent of cases in adults and children will present differential problems Of the fungus infections, coccidioidomycosis and histoplasmosis are most apt to cause difficulty, since these infections frequently occur without concomitant cutaneous involvement A history of residence in an endemic area is helpful in these cases Most of the rickettsial infections except Q fever can be differentiated by the presence of an eschar or the development of a rash However, Q fever may closely resemble primary atypical pneumonia, although in general the onset is more sudden, constitutional symptoms predominate throughout the illness, respiratory symptoms are minimal, and the roentgenographic evidence of infiltration is usually more focal in character and more apt to appear in the lung parenchyma without hilar involvement Although pulmonary infiltration may occur in such contagious diseases as measles and chickenpox, the commonest viral diseases that should be considered in differential diagnosis are those due to the psittacosis group of viruses, the influenza viruses including newborn pneumonitis virus, type Sendai, the virus of lymphocytic choriomeningitis, and the adenoviruses, particularly those types (4 and 7) that most frequently cause acute respiratory disease (ARD) of recruits An epidemiologic history of exposure to birds or mice suggests the possibility of psittacosis or lymphocytic choriomeningitis. Cases due to one of the influenza viruses or to adenoviruses are most apt to occur during epidemics of these diseases Rarely, toxoplasmosis and ascariasis or other parasitic diseases must be considered in the differential diagnosis

In general, the final diagnosis of the above infections can be established by isolation of the infecting agent, by the demonstration of a rise in serum antibodies during convalescence, or by both procedures Further differential features of the viral and rickettsial diseases that may present difficulty are discussed in other chapters of this book and will not be considered further here

Noninfectious processes resembling primary atypical pneumonia, such as bronchiectasis, eosinophilic pneumonia or Loeffler's syndrome, sarcoidosis carcinoma, atelectasis and infarction usually can be recognized and differentiated by clinical and laboratory procedures and the course of illness

TREATMENT

Symptomatic and supportive treatment forms the basis for the management of patients with primary atypical pneumonia As in other infectious diseases, bed rest, good nursing care, a palatable diet suited to the patient's desires, and adequate parenteral or oral fluids are important Drugs or procedures such as Neo-synephrine, codeine, dihydrocodeinone, oxygen and postural drainage may be helpful Antipyretic drugs with a diaphoretic action, such as acetylsalicylic acid, should be avoided if sweating is a prominent feature. Sponge baths with cool water or alcohol should be avoided in severely ill patients, because a hemolytic crisis may be induced if the titer of cold hemagglutinins in the patient's serum is high. Sedation and transfusion may be employed if indicated.

the development of cold hemagglutinins but also agglutinins for streptococcus MG, complement-fixing antibodies for a variety of antigens, and other peculiar behavior Horsfall et al (1943), for example, showed that sputum from 1 patient with primary atypical pneumonia, when inoculated intranasally in cotton rats, induced pulmonary consolidation and stimulated the development of neutralizing antibodies for pneumonia virus of mice (PVM) and that mixing the sputum with convalescent sera from human cases could prevent both the development of pneumonia and such antibody production The variety of reactions occurring in the sera of patients suggests that antibodies of several sorts may be induced nonspecifically in some manner as yet unknown Thus it may be that the agent isolated by Eaton and by Liu and the antibody reactions to it demonstrated by the indirect fluorescent antibody technic represent still another example of a peculiar reaction not necessarily dependent solely on the specific etiologic agent Further studies and data are necessary before the puzzling etiology of primary atypical pneumonia is clarified.

DIAGNOSIS

The diagnosis of primary atypical pneumonia is based on a process of exclusion, since direct confirmation is not now possible by means of a practical and specific laboratory procedure utilizing the causative agent. Two serologic tests have been used extensively as retrospective aids in diagnosis· cold hemagglutination and agglutination of streptococcus MG The technics are described in Chapter 10, "Serologic Reactions in Viral and Rickettsial Infections" Both tests are best performed with acute-phase serum specimens obtained during the first week of illness and convalescent-phase specimens obtained 3 or 4 weeks after onset If either or both types of agglutinin appear in the convalescent sera, and especially if a 4-fold or greater increase in titer is demonstrable some weeks after onset, there is a high probability that the diagnosis is correct The absence of these agglutinins does not exclude the diagnosis but makes it more difficult to establish

Cold hemagglutinins for group O human erythrocytes appear in the convalescent sera of approximately 50 per cent of the patients, with variations in different series of cases from about 30 per cent to almost 100 per cent. Maximum titers are usually reached in the 3d or the 4th week after onset, following which a decline occurs during the succeeding 2 or 3 weeks. Agglutinins for streptococcus MG have been reported in some 20 to 75 per cent of cases. These agglutinins usually appear in the 2d or the 3d week after onset, reach their maximum titers in the 4th or the 5th week and begin to decline in the 7th or the 8th week There is a positive correlation between the frequency with which both of these agglutinins develop and the severity of the disease, the extent of pulmonary involvement and the duration of illness A similar correlation is found in the height of the titers reached.

The indirect fluorescent antibody technic of Liu (1957), using the Eaton virus, may also be employed with acute-phase and convalescent sera to substantiate the diagnosis, although sufficient numbers of cases have not yet been studied to give an indication of the frequency with which a positive reaction will occur This technic may also be employed to detect the presence of the Eaton virus in chick embryos inoculated with sputum or throat washings obtained from patients during the acute phase of their illnesses, and the results may be determined within a week after inoculation of the eggs However, the technic is highly specialized, and it is unlikely that it will be utilized in more than a few medical centers for some time to come The value of any of the above tests in the management of the patient is obviously limited, since the acute phase of the illness, when decisions regarding therapy must be made, will be over in most instances before the results of the tests are known

Apart from primary atypical pneumonia, cold hemagglutinins may be found in patients with trypansomiasis and occasionally in patients with malaria, blood dyscrasias, or liver disease Agglutinins for streptococcus MG are rarely found except in patients with primary atypical pneumonia The specificity of the new fluorescent antibody test for Eaton's virus has not yet been adequately determined, but no such antibody has been detected in the convalescent sera of a limited number

and by transmission studies in man. However, such a relationship cannot be confirmed until specific diagnosis and detection of all cases are possible The incidence of the disease usually rises during the winter months, so that an etiologic relationship between this disease and other respiratory infections may be more apparent than real All age groups are attacked. The degree and the duration of immunity following infection are not known, but second attacks have been observed

CONTROL MEASURES

No specific prophylactic measure is available. With the sporadic cases, case-to-case spread is rarely apparent, and isolation of the patients is seldom practiced Isolation precautions may be helpful in the event of a spreading outbreak

REFERENCES

(For complete references, see also 2nd edition, chapter 17)

Adams, J M, 1945, Third epidemic of primary virus pneumonitis among infants in Minnesota, Journal-Lancet *65*, 192-193

Adams, J M, Green, R G, Evans, C A, and Beach, N, 1942, Primary virus pneumonitis A comparative study of two epidemics J Pediat *20*, 405-420

Arakawa, S, Kondo, I, Kaneko, T, and Goto, N, 1956, Studies on primary atypical pneumonia virus, Arch Virusforsch *7*, 88-109

Blake, F G, Howard, M E, and Tatlock, H, 1942, Feline virus pneumonia and its possible relation to some cases of primary atypical pneumonia in man, Yale J Biol & Med *15*, 139

Campbell, T A, Strong, P S, Grier, G S, and Lutz, R J, 1943, Primary atypical pneumonia A report of 200 cases at Fort Eustis, Virginia J.A.M.A *122*, 723-729

Commission on Acute Respiratory Diseases in collaboration with Dammin, G J, and Weller, T H, 1945, Attempts to transmit primary atypical pneumonia and other respiratory tract infections to the mongoose, J Immunol *50*, 107-114

Commission on Acute Respiratory Diseases, 1946, The transmission of primary atypical pneumonia to human volunteers I Experimental methods II Results of inoculation III Clinical features IV Laboratory studies Bull Johns Hopkins Hosp *79*, 97-167,

Commission on Acute Respiratory Diseases, 1947, Experimental transmission of minor respiratory illness to human volunteers by filter passing agents I Demonstration of two types of illness characterized by long and short periods and different clinical pictures J Clin Invest *26*, 957-973

Commission on Acute Respiratory Diseases, 1947, Experimental transmission of minor respiratory illness to human volunteers by filter-passing agents II Immunity on reinoculation with agents from the two types of minor respiratory illness and from primary atypical pneumonia J Clin Invest. *26*, 974-982

Curnen, E C, Mirick, G S, Ziegler, J E, Jr, Thomas, L, and Horsfall, F L, Jr, 1945, Studies on primary atypical pneumonia I Clinical features and results of laboratory investigations J Clin Invest *24*, 209-226

Dingle, J H, et al, 1944, Primary atypical pneumonia, etiology unknown, Am J Hyg *39*, 67-128, 197-268, 269-336

Dingle, J H, and Finland, M, 1942, Virus pneumonias II Primary atypical pneumonias of unknown etiology New England J Med *227*, 378-385

Eaton, M D, 1950, Virus pneumonia and Pneumonitis Viruses of Man and Animals Handbuch der Virusforschung, pp 87-140, Vienna, Springer

Eaton, M D, Meiklejohn, G, Van Herick, W, 1944, Studies on the etiology of primary atypical pneumonia A filterable agent transmissible to cotton rats, hamsters, and chick embryos J Exper Med *79*, 649 668

Favour, C B, 1944, Infections associated with an epidemic of primary interstitial pneumonia, New England J Med *230*, 537-541

Finland, M, 1952, Antimicrobial treatment for viral and related infections I Antibiotic treatment of primary atypical pneumonia New England J Med *247*, 317-325

Gallagher, J R, 1934, Bronchopneumonia in adolescents. Yale J Biol & Med *7*, 23-40

Ginsberg, H S, Badger, G F, Dingle, J H, Jordan, W S, Jr, and Katz, S, 1955, Etiologic Relationship of the RI-67 agent to "Acute Respiratory Disease (ARD)" J Clin Invest *34*, 820-831

Golden, A, 1944, Pathologic anatomy of "Atypical Pneumonia, etiology undetermined," Arch Path *38*, 187-202

Horsfall, F L, Jr, Curnen, E C, Mirick, G S, Thomas, L, and Ziegler, J E, Jr, 1943, A virus recovered from patients with primary atypical pneumonia, Science *97*, 289-291

Jensen, K E, Minuse, E, and Ackermann, W W, 1955, Serologic evidence of American experience with newborn pneumonitis virus (type Sendai) J Immunol *75*, 71-77

Jordan, W S, Jr, 1949, The infectiousness and incubation period of primary atypical pneumonia, Am J Hyg *50*, 315-330

Jordan, W S, Jr, Albright, R W, McCain, F H, and Dingle, J H, 1951, Clinical variations in primary atypical pneumonia, Am J Med *10*, 3-20

Kneeland, Y, and Smetana, H F, 1940, Current bronchopneumonia of unusual character and undetermined etiology, Bull Johns Hopkins Hosp. *67*, 229-267

Kuroya, M., and Ishide, W, 1953, Newborn virus pneumonitis (type Sendai) II Isolation of new virus possessing hemagglutinin activity Yokohama M Bull *4*, 217-233

Complications should be treated as they arise. Secondary bacterial infection occurs extremely rarely, when it does, it is accompanied by a dramatic change in the clinical condition of the patient, evidenced by manifestations of sepsis such as spiking fever, prostration and elevation of the leukocyte count Therapeutic agents should be selected in accordance with the sensitivity of the infecting bacterium Convalescence may be prolonged due to asthenia and slow resolution of the pulmonary infiltration, but activity need be restricted only to the tolerance of the patient

A variety of substances have been employed in the search for a specific therapeutic agent for this disease However, interpretation of the results has been difficult, not only in individual cases but also in controlled studies because of the variation in the course of the disease from patient to patient. The sulfonamide drugs, penicillin and streptomycin are now considered to be without beneficial effect The situation with respect to the so-called broad-spectrum antibiotics, such as the tetracyclines and chloramphenicol, is not so clear, and there are several conflicting reports regarding their efficacy. Many of the reports are based on dramatic improvement in individual patients after administration of a drug Other studies comparing treated and untreated groups or control groups given penicillin have shown apparently beneficial results in some series but not in others (Tillotson and Oseasohn, 1952, Finland, 1952). It may be that the same diseases were not being treated in the different studies, as perhaps is indicated by the fact that differing proportions of the patients in the various series have shown the development of cold hemagglutinins. Meiklejohn et al (1954) have reported that patients who have severe illnesses with temperatures above 102° F. are benefited to the greatest extent but that similar benefit is not observed in the milder cases. Whether this result is due to a specific action of the antibiotic on the virus or to an antipyretic action (Tillotson and Ginsberg, 1954) has not yet been clearly established In this respect it is interesting that streptococcus MG is resistant to sulfonamide drugs but is susceptible to penicillin and chlortetracycline. The virus of Eaton is resistant to penicillin and chloramphenicol but is susceptible to streptomycin and chlortetracycline These findings do not appear at present to be compatible with the evaluations of therapy in patients.

In view of the rarity of secondary bacterial infections, the prophylactic use of any chemotherapeutic agent during the course of the illness is not warranted

EPIDEMIOLOGY

The disease occurs throughout the world, usually in endemic form. Its epidemic occurrence in both civilian groups (Gallagher, 1934, Reimann, 1938; Kneeland and Smetana, 1940; Favour, 1944, Jordan, 1949), such as hospital personnel and families, and in military populations (Campbell et al, 1943; Dingle et al, 1944) has been described frequently and was one of the factors responsible for the recognition of this form of pneumonia. Yet few epidemics have occurred in recent years, and the cases observed since 1948 have been small in number and mild in nature Since the distribution of antibody to the etiologic agent in the general population cannot yet be measured, past behavior of the disease is crudely defined, and the likelihood of future epidemics is totally unpredictable.

The true incidence is not known. Among armed forces personnel during World War II, the attack rate averaged 10 per 1,000 per year, about 10 times the incidence of bacterial pneumonia In some epidemics, attack rates as high as 15 to 20 per cent have been reported, and in one study of multiple cases in families a rate of 35 per cent was noted (Jordan, 1949). Case-to-case spread has been apparent in such instances, and the epidemiologic behavior has suggested a relationship between degree of contact and contagiousness The mechanism of transmission appears to be through direct contact with infected persons These inferences are supported by the results of studies in volunteers (Commission on Acute Respiratory Diseases, 1946) which indicated that the infection may be transmitted by respiratory secretions and that the portal of entry is the upper respiratory tract The duration of the period of communicability is unknown

An etiologic association between primary atypical pneumonia and certain minor respiratory illnesses has been suggested by the sequence of occurrence of cases in epidemics

and by transmission studies in man However, such a relationship cannot be confirmed until specific diagnosis and detection of all cases are possible. The incidence of the disease usually rises during the winter months, so that an etiologic relationship between this disease and other respiratory infections may be more apparent than real All age groups are attacked The degree and the duration of immunity following infection are not known, but second attacks have been observed

CONTROL MEASURES

No specific prophylactic measure is available With the sporadic cases, case-to-case spread is rarely apparent, and isolation of the patients is seldom practiced Isolation precautions may be helpful in the event of a spreading outbreak

REFERENCES

(*For complete references, see also 2nd edition, chapter 17*)

Adams, J M, 1945, Third epidemic of primary virus pneumonitis among infants in Minnesota, Journal-Lancet *65*, 192-190

Adams, J M, Green, R G, Evans, C A, and Beach, N, 1942, Primary virus pneumonitis A comparative study of two epidemics *J Pediat* *20*, 405-420

Arakawa, S, Kondo, I, Kaneko, T, and Goto, N, 1956, Studies on primary atypical pneumonia virus, Arch Virusforsch *7*, 88 109

Blake, F G, Howard, M E, and Tatlock, H, 1942, Feline virus pneumonia and its possible relation to some cases of primary atypical pneumonia in man, Yale J Biol & Med *15*, 139

Campbell, T A, Strong, P S, Grier, G S, and Lutz, R J, 1943, Primary atypical pneumonia A report of 200 cases at Fort Eustis, Virginia J.A.M.A *122*, 723-729

Commission on Acute Respiratory Diseases in collaboration with Dammin, G J, and Weller, T H, 1945, Attempts to transmit primary atypical pneumonia and other respiratory tract infections to the mongoose, *J Immunol* *50*, 107-114

Commission on Acute Respiratory Diseases, 1946, The transmission of primary atypical pneumonia to human volunteers I Experimental methods II Results of inoculation III Clinical features IV Laboratory studies Bull Johns Hopkins Hosp *79*, 97-167

Commission on Acute Respiratory Diseases, 1947, Experimental transmission of minor respiratory illness to human volunteers by filter passing agents I Demonstration of two types of illness characterized by long and short periods and different clinical pictures J Clin Invest *26*, 957-973

Commission on Acute Respiratory Diseases, 1947, Experimental transmission of minor respiratory illness to human volunteers by filter-passing agents II Immunity on reinoculation with agents from the two types of minor respiratory illness and from primary atypical pneumonia J Clin Invest *26*, 974-982

Curnen, E C, Mirick, G S, Ziegler, J E, Jr, Thomas, L, and Horsfall, F L, Jr, 1945, Studies on primary atypical pneumonia I Clinical features and results of laboratory investigations J Clin Invest *24*, 209-226

Dingle, J H, et al, 1944, Primary atypical pneumonia, etiology unknown, Am J Hyg *39*, 67-128, 197-268, 269-336

Dingle, J H, and Finland, M, 1942, Virus pneumonias II Primary atypical pneumonias of unknown etiology New England J Med *227*, 378-385

Eaton, M D, 1950, Virus pneumonia and Pneumonitis Viruses of Man and Animals Handbuch der Virusforschung, pp 87-140, Vienna, Springer

Eaton, M D, Meiklejohn, G, Van Herick, W, 1944, Studies on the etiology of primary atypical pneumonia A filterable agent transmissible to cotton rats, hamsters, and chick embryos J Exper Med *79*, 649-668

Favour, C B, 1944, Infections associated with an epidemic of primary interstitial pneumonia, New England J Med *230*, 537-541

Finland, M, 1952, Antimicrobial treatment for viral and related infections I Antibiotic treatment of primary atypical pneumonia New England J Med *247*, 317-325

Gallagher, J R, 1934, Bronchopneumonia in adolescents, Yale J Biol & Med *7*, 23-40

Ginsberg, H S, Badger, G F, Dingle, J H, Jordan, W S, Jr, and Katz, S, 1955, Etiologic Relationship of the RI-67 agent to "Acute Respiratory Disease (ARD)" J Clin Invest *34*, 820-831

Golden, A, 1944, Pathologic anatomy of "Atypical Pneumonia, etiology undetermined," Arch Path *38*, 187-202

Horsfall, F L, Jr, Curnen, E C, Mirick, G S, Thomas, L, and Ziegler, J E, Jr, 1943, A virus recovered from patients with primary atypical pneumonia, Science *97*, 289-291

Jensen, K E, Minuse, E, and Ackermann, W W, 1955, Serologic evidence of American experience with newborn pneumonitis virus (type Sendai) J Immunol *75*, 71-77

Jordan, W S, Jr, 1949, The infectiousness and incubation period of primary atypical pneumonia, Am J Hyg *50*, 315-330

Jordan, W S, Jr, Albright, R W, McCain, F H, and Dingle, J H, 1951, Clinical variations in primary atypical pneumonia, Am J Med *10*, 3-20

Kneeland, Y, and Smetana, H F, 1940, Current bronchopneumonia of unusual character and undetermined etiology, Bull Johns Hopkins Hosp *67*, 229-267

Kuroya, M, and Ishide, W, 1953, Newborn virus pneumonitis (type Sendai) II Isolation of new virus possessing hemagglutinin activity Yokohama M Bull *4*, 217-233

Liu, C, 1957, Studies on primary atypical pneumonia I Localization, isolation and cultivation of the virus in chick embryos J Exper Med *106*, 455-466

Meiklejohn, G, Thalman, W G, Waligora, D J, Kempe, C H, and Lennette, E H, 1954, Chemotherapy of primary atypical pneumonia, J A M A *154*, 553-557

Parker, F, Jr, Jolliffe, L S, and Finland, M, 1947, Primary atypical pneumonia Report of eight cases with autopsies Arch Path *44*, 581-608

Reimann, H A, 1938, An acute infection of the respiratory tract with atypical pneumonia A disease entity probably caused by a filtrable virus J A M A *3*, 2377-2384.

Stokes, J, Jr, Kenney, A S, and Shaw, D R, 1939, A new filtrable agent associated with respiratory infections, Tr & Stud Coll Physicians Philadelphia *6*, 329-333

Thomas, L, Mirick, G S, Curnen, E C, Ziegler, J E, Jr, and Horsfall, F L, Jr, 1945, Studies on primary atypical pneumonia II Observations concerning the relationship of a non-hemolytic streptococcus to the disease J. Clin Invest *24*, 227-240

Tillotson, I G, and Ginsberg, H S, 1954, An antipyretic action of aureomycin Inhibition of febrile response to influenza virus in rabbits Proc Soc Exper Biol & Med *85*, 192-197

Tillotson, I G, and Oseasohn, R O, 1952, The treatment of primary atypical pneumonia—the need for control in the evaluation of aureomycin therapy, Ohio M J *48*, 32-33

Weir, J M, and Horsfall, F L, Jr, 1940, The recovery from patients with acute pneumonitis of a virus causing pneumonia in the mongoose, J Exper Med *72*, 595-610

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30

The Adenovirus Group

INTRODUCTION

The adenovirus group is composed of at least 23 immunologically distinct types of viruses that are related by a common complement-fixing antigen and possess the properties of ether resistance, stability to temperature and pH changes, lack of pathogenicity for laboratory animals, and multiplication in a number of lines of epithelial or epithelial-like cells in tissue culture. To date 18 types have been isolated from human and 5 from simian sources. Certain of these agents, particularly types 3, 4 and 7, have been associated with both epidemics and sporadic cases of respiratory disease. The type 8 virus appears to be the major causative agent of epidemic keratoconjunctivitis. Numerous types have been isolated from surgically removed tonsils and adenoids, some of these types have not yet been shown to cause disease

HISTORY

The first isolations to be reported were made by Rowe et al. (1953) from fragments of human adenoids grown in tissue culture After prolonged incubation cytopathic change was noted in the epithelial-like cells of some of the cultures and passage revealed the presence of virus A large number of strains, principally of types 1, 2 and 5, have since been obtained from cultures of both tonsils and adenoids when grown under similar conditions These agents, initially called *adenoid degeneration* or *AD* agents, had no apparent relation to respiratory disease. Almost simultaneously, Hilleman and Werner (1954) isolated by tissue culture technics a cytopathogenic agent from army recruits sick with an influenzalike or grippelike illness. An increase in neutralizing and complement-fixing antibodies against the virus was shown to occur in the patients Previously, this disease had been described by several investigators as an important problem in military forces and had been variously termed catarrhal fever, febrile catarrh and acute respiratory disease of recruits (ARD). During World War II the Commission on Acute Respiratory Diseases (1947a, b, c) presented evidence that acute respiratory disease (ARD) was a distinct clinical entity caused by a virus Retrospective studies employing sera still available from the Commission's volunteer experiments in 1947 indicated that the virus isolated by Hilleman and Werner (designated *RI-67*) was identical with or immunologically closely related to the virus which was transmitted to the volunteers (Ginsberg et al , 1955a) [illegible]

[illegible] Ginsberg et al , 1955b)

Huebner et al (1954) showed that at least 6 immunologically distinct types of virus existed, all related by a common group-specific complement-fixing antigen, and suggested the name adenoidal-pharyngeal-conjunctival (APC) viruses for the group The RI-67 virus isolated by Hilleman and Werner was demonstrated to be a member of

Liu, C, 1957, Studies on primary atypical pneumonia I Localization, isolation and cultivation of the virus in chick embryos J Exper Med *106*, 455-466

Meiklejohn, G, Thalman, W G, Waligora, D J, Kempe, C H, and Lennette, E H, 1954, Chemotherapy of primary atypical pneumonia, J.A.M.A *154*, 553-557

Parker, F, Jr, Jolliffe, L S, and Finland, M, 1947, Primary atypical pneumonia Report of eight cases with autopsies Arch Path *44*, 581-608

Reimann, H A, 1938, An acute infection of the respiratory tract with atypical pneumonia A disease entity probably caused by a filtrable virus J A M A *3*, 2377-2384

Stokes, J, Jr, Kenney, A S, and Shaw, D R, 1939, A new filtrable agent associated with respiratory infections, Tr & Stud Coll Physicians Philadelphia *6*, 329-333

Thomas, L, Mirick, G S, Curnen, E C, Ziegler, J E, Jr, and Horsfall, F L, Jr, 1945, Studies on primary atypical pneumonia II Observations concerning the relationship of a non-hemolytic streptococcus to the disease J Clin Invest *24*, 227-240

Tillotson, I G, and Ginsberg, H S, 1954, An antipyretic action of aureomycin Inhibition of febrile response to influenza virus in rabbits Proc Soc Exper Biol & Med *85*, 192-197

Tillotson, I G, and Oseasohn, R O, 1952, The treatment of primary atypical pneumonia—the need for control in the evaluation of aureomycin therapy, Ohio M J *48*, 32-33

Weir, J M, and Horsfall, F L, Jr, 1940, The recovery from patients with acute pneumonitis of a virus causing pneumonia in the mongoose, J Exper Med *72*, 595-610

mal limits, and no abnormalities are found in the urine Cultures of the throat or of the sputum, if available, reveal only the normal bacterial flora of the respiratory tract The febrile course is short, generally lasting from 2 to 4 days The fever may reach levels of 103° to 104°, but the average maximum temperature is about 100° F The fever may subside abruptly or by lysis. Constitutional symptoms subside with defervescence, but symptoms referable to the respiratory tract, when present, tend to persist for from 1 to 2 weeks Recovery is uneventful and complete, and bacterial complications have not been described among hospitalized patients

PHARYNGITIS AND PHARYNGOCONJUNCTIVAL FEVER

(SYNONYMS Nonbacterial pharyngitis, nonstreptococcal exudative tonsillitis and pharyngitis)

The clinical picture of pharyngitis and pharyngoconjunctival fever due to the adenoviruses is remarkably similar to that described above for acute respiratory disease (ARD) when exception is made of those symptoms and physical signs which are related to the local inflammation in the throat and in some cases of the eye The incubation period is estimated to be 5 to 7 days (Bell et al, 1955) The onset is gradual in about 80 per cent of the cases The illness is characterized by fever, sore throat and in some patients by conjunctivitis The symptoms of feverishness, chilliness, headache, anorexia, sore throat, hoarseness and cough are present in about three fourths of the patients Malaise is not uncommon In less than half the patients the cough may be associated with sputum and dull substernal chest pain On physical examination the patients appear to be acutely but not seriously ill Fever is usually present, and the pulse rate is elevated in accordance with it When present, conjunctivitis is mild and follicular in type and may be unilateral or bilateral Nasal obstruction and slight coryza are not uncommon The pharynx may be normal or moderately red, and lymphoid hyperplasia is common Exudate may or may not be present on the posterior pharyngeal wall and on the tonsils When present it is not extensive, is often pinpoint, grayish white in appearance and frequently is confined to the pharyngeal wall Redness and edema of the palate and petechiae may rarely be found. Moderate cervical lymphadenopathy may be present and in patients with conjunctivitis there may be nontender, preauricular lymphadenopathy. The total leukocyte count may be normal or slightly elevated but rarely exceeds 12,000 cells per cu mm The differential count may show a mild neutrophilia The urine is normal, and cultures of the throat reveal the common respiratory tract flora During the acute stage of illness virus may be isolated from the throat and when conjunctivitis is present, from the conjunctivae

The febrile illness is seldom longer than 4 or 5 days in duration The fever is sustained and sometimes attains levels of 103° or 104° F The average peak of temperature is between 101° and 102° F, and the fever ordinarily declines by lysis Sore throat and constitutional symptoms predominate at the onset, with other symptoms referable to the respiratory tract becoming more prominent as the illness progresses Sore throat is generally mild Nausea, vomiting, diarrhea, epistaxis and transient hepatomegaly have been noted rarely When conjunctivitis is present, it may last for a few days to 3 weeks Photophobia and retro-orbital pain have not been associated with the conjunctivitis, although corneal opacities have been reported (Cockburn et al, 1956, Ormsby and Aitchison, 1955) Otitis media may occur as a complication or as part of the disease Recovery is complete without sequelae, and no fatalities have been reported Illnesses similar to the spontaneously occurring disease have been induced by the inoculation of volunteers In view of the fact that various types of adenovirus may be isolated from tonsillar and adenoidal tissue removed surgically, Huebner et al (1954) have postulated that these agents may cause a low-grade chronic infection of the adenoidal tissue leading to hypertrophy Sufficient data are not yet available to substantiate this view

CONJUNCTIVITIS AND KERATOCONJUNCTIVITIS

Follicular conjunctivitis, caused by one of several types of adenovirus, may occur as the

this group and was assigned the designation type 4. In addition, an agent isolated by Neva and Enders (1954) was shown to be a strain of type 3. Due to the differences in nomenclature employed by the various laboratories studying these viruses, a group of interested investigators met and agreed upon the name *Adenoviruses* (Enders et al., 1956) as being generally acceptable and in accordance with the recommendations of the Subcommittee on Viruses of the International Nomenclature Committee.

With time, excellent clinical, epidemiologic and laboratory data have accumulated to indicate that type 3 virus is a frequent etiologic agent of nonbacterial pharyngitis with or without conjunctivitis (Parrott et al., 1954; Bell et al., 1955; Ginsberg et al., 1955b, Cockburn et al., 1956; Jawetz et al., 1956), that types 4, 7 and 14 are responsible for acute respiratory disease (ARD) (Hilleman et al., 1955d, Berge et al., 1955, Andrews et al., 1956, Dascomb and Hilleman, 1956, Rowe et al., 1956a, van der Veen and Kok, 1957) and for some cases of nonbacterial pharyngitis, and that type 8 virus is probably the causative agent of epidemic keratoconjunctivitis (Jawetz et al., 1955a) Type 8 adenovirus is not immunologically or biologically related to St Louis encephalitis virus, an agent closely related to or identical with that reported by Sanders and Alexander (1943) to be the etiologic agent of epidemic keratoconjunctivitis (Cheever, 1951, Ruchman, 1951). Since neither the original viruses nor the sera from the patients studied by Sanders and Alexander are available, it probably will not be possible to compare the type 8 adenovirus with the agent isolated by these workers.

CLINICAL PICTURE

The clinical picture of infections caused by the adenoviruses can be presented most conveniently here as comprising 3 syndromes: (1) acute respiratory disease (ARD), occurring almost exclusively in military recruits and caused predominantly by types 4 and 7, (2) pharyngitis and pharyngoconjunctival fever, caused principally by type 3, and (3) conjunctivitis and keratoconjunctivitis, caused primarily by types 3 and 8, respectively. However, there is considerable overlapping in the syndromes, so that one type of virus may cause an illness resembling acute respiratory disease in one patient and conjunctivitis or pharyngoconjunctival fever in another. This variation is particularly evident in sporadic cases, whereas the clinical picture is more consistent in epidemics. It should be emphasized that these clinical entities may also be caused by other agents and so are not necessarily specific for the adenovirus group.

ACUTE RESPIRATORY DISEASE (ARD)

(SYNONYMS: Febrile catarrh, acute respiratory disease of recruits, catarrhal fever, common respiratory disease)

This disease, which has been described almost exclusively in military recruits, is usually a relatively mild, grippelike illness. The incubation period, as indicated epidemiologically and from transmission studies in volunteers, is from 5 to 6 days. Ordinarily, the onset is gradual over a period of 2 to 3 days, but occasionally it may be abrupt. Feverishness and chilliness are the outstanding constitutional symptoms and usually are accompanied by headache, malaise and anorexia. Symptoms referable to the respiratory tract may appear early but generally are less prominent than the constitutional symptoms. Nasal obstruction and occasionally slight nasal discharge occur in approximately half of the patients. Irritated or sore throat is common but is mild in degree. Minimal hoarseness and dry cough are common, although fewer than half the patients have a productive cough or pain in the chest. On physical examination the patient appears to be acutely but rarely severely ill. Physical signs may be few or absent and usually are noted in less than half the patients. Nasal obstruction, mild injection of the pharynx and the palate, and lymphoid hyperplasia on the pharyngeal wall are found most frequently. Edema of the mucous membranes and mild cervical adenopathy are present in approximately 10 per cent of the patients. Pulmonary rales and roentgenographic evidence of infiltration of the lungs may be found in approximately 10 to 12 per cent of the patients, particularly during epidemics. The pulse rate is ordinarily compatible with the temperature, and the respiratory rate is normal. Total leukocyte and differential counts are usually within nor-

formation of type-specific antibodies following intranasal inoculation of a small dose of virus The animals to which adenovirus infection could not be transmitted were infant and adult mice, and infant hamsters inoculated by intranasal, intraperitoneal, intracerebral and intravenous routes, adult guinea pigs inoculated intraperitoneally and intranasally as well as on the cornea and in the anterior chamber of the eye, adult cotton rats by intracerebral and intranasal routes, on the conjunctiva and in the cornea, white rats inoculated intracerebrally and intranasally, rhesus monkeys inoculated intracerebrally, intranasally and subcutaneously, kittens inoculated intracerebrally adult ferrets inoculated by the intracerebral, the intraperitoneal and the intranasal routes and embryonated chicken eggs inoculated into the yolk, the allantoic and the amniotic sacs However, tissue culture technics offer a large number of cell lines which support multiplication of adenoviruses and respond to infection with characteristic cytopathic changes HeLa cells epithelial-like cells derived by Grey from an epidermoid carcinoma of the cervix, have proved to be most generally useful for propagation of adenoviruses In addition these agents multiply and induce cytopathic changes in a variety of other epithelial-like cell lines derived from several types of cancer, human conjunctival membrane nasal mucosa, bone marrow, tonsils, adenoids, liver and intestine, and maintained in continuous culture Adenoviruses will also multiply in tissue cultures prepared from human tracheal epithelium, human amnion, monkey and rabbit kidney and rabbit tracheal epithelium Undoubtedly many other susceptible cells have been employed, or will be found to support multiplication of these agents Although epithelial cells appear to be most susceptible to infection, fibroblasts in mixed cultures also are affected eventually Whether adenoviruses propagate in these cells or damage the fibroblasts by a toxic action has not been determined

The only animal shown to be readily susceptible to adenovirus infection is man The Commission on Acute Respiratory Diseases (1947b, c) produced acute respiratory disease (ARD) by inhalation of a spray of filtered respiratory secretions from a characteristic case of acute respiratory disease (ARD). Subsequent studies indicated that the virus transmitted was identical with or immunologically closely related to type 4 virus (Ginsberg et al, 1955a). When inoculated intranasally into volunteers, type 1, 2, 3 or 4 adenovirus, previously passed in HeLa cells, resulted in the formation of neutralizing and complement-fixing antibodies but not in the production of clinical respiratory disease (Bell et al, 1956b, Southam et al., 1956) On the other hand, type 1 virus propagated in embryonic human kidney cells, when inoculated intranasally, produced pharyngitis in 2 of 4 volunteers who had no pre-existing neutralizing antibodies (Roden et al, 1956) When virus which had been grown in monkey kidney cells was rubbed on the conjunctiva, moderately severe catarrhal conjunctivitis and pharyngitis accompanied by systemic symptoms resulted (Bell et al, 1956b) Adenovirus type 4 or 7 injected intramuscularly by Hilleman et al (1957b) induced fever, constitutional symptoms and the production of antibodies, but multiplication of virus was not demonstrated Type 8 virus, when instilled into the conjunctival sac after scraping of the conjunctiva or the cornea, resulted in follicular conjunctivitis, keratitis and corneal opacities characteristic of epidemic keratoconjunctivitis (Mitsui et al, 1957, Bietti and Bruna, 1957), and infection spread to susceptible contacts However, instillation of type 8 virus without injury to the conjunctiva or the cornea did not initiate disease.

At least 5 serologic types are apparently of simian origin Four of these types were obtained from monkeys, and one was isolated from the stool of a chimpanzee with a mild upper respiratory disease (Rowe et al, 1956b, 1958) It has not yet been determined whether disease can be initiated in monkeys or chimpanzees by inoculation of these types

ETIOLOGY

The adenovirus group is composed of a number of viruses related by a common complement-fixing antigen and many similar biologic properties Although the small antigenic particles detected by complement-fixation technics are immunologically similar but probably not identical for all viral types

only manifestation of infection or, as indicated above, may be associated with involvement of the respiratory tract and with systemic manifestations. Involvement of one or both eyes may occur and is manifested by redness of both the bulbar and the palpebral conjunctivae. Excess lacrimation, serous exudation and occasionally edema may occur. Other manifestations of ocular disease are usually lacking, although corneal opacities have been reported (Cockburn et al., 1956, Ormsby and Aitchison, 1955). The clinical disease has been reproduced in volunteers by swabbing the lower palpebral conjunctivae with adenovirus types 1, 3, 4 and 5 (Bell et al., 1956b). Conjunctivitis developed within 2 to 7 days and persisted for varying periods of time up to 3 weeks. Preauricular lymphadenopathy, which may or may not be tender, is commonly found. Recovery is complete without sequelae.

The clinical picture of epidemic keratoconjunctivitis is now becoming clarified by recent evidence that the disease is associated with or caused by adenovirus type 8 (Jawetz et al., 1955b; 1957). The incubation period of the naturally occurring infection is unknown, but it has averaged 5 to 7 days in volunteers inoculated with material from patients with the disease (Aoki and Kasahara, 1941) or with type 8 virus grown in tissue culture (Mitsui et al., 1957). The onset is sudden, with redness and chemosis of the conjunctiva and edema of the periorbital tissues. Exudate may be absent or may be serous or seromucoid in character. Constitutional manifestations of low-grade fever, headache and malaise may be present. Initially, the disease is ordinarily unilateral, but in about half the patients the other eye becomes involved 3 to 7 days later. Within 2 to 4 days after onset, preauricular lymphadenopathy develops and is usually tender. Small, round, superficial opacities of the cornea are noted within 4 to 14 days after onset. Ulceration of the cornea does not occur. In severe cases these lesions may coalesce. The duration of illness is from 2 to 4 weeks, and complete recovery occurs in most patients. However, keratitis with impairment of vision may persist in from 1 to 10 per cent of the patients.

PATHOLOGIC PICTURE

Deaths as a result of the most common adenovirus infections have not been reported. Hence, the pathology of acute respiratory and ocular infections, with the exception of epidemic keratoconjunctivitis, has not been described. However, 2 fatal cases of nonbacterial pneumonia in infants from which adenoviruses were isolated have been reported (Drouhet, 1957; Chany et al., 1958). From one of these, type 7a virus was isolated (Chany et al., 1958). The exact role of the adenovirus in the causation of the marked pulmonary consolidation cannot be assessed. However, a striking finding in the bronchi and the alveolar walls was the presence of numerous epithelial cells in which the nuclei were enlarged and contained a central basophilic mass surrounded by a vacuolated area. In some cells the intranuclear inclusion appeared to be eosinophilic. The nuclear changes observed were very similar in appearance to the rosette form of the late stages of cytologic alterations described in adenovirus-infected cells in culture (Barski, 1956; Boyer et al., 1957a).

Epidemic keratoconjunctivitis is characterized by marked edema of the conjunctivae followed by an exudate of mononuclear cells, chiefly lymphocytes. These cells, along with epithelial cells, may be seen in smears and scrapings from the conjunctivae. On the third to the fifth day of disease a pseudomembrane containing many mononuclear and degenerating epithelial cells may cover the conjunctivae. On about the sixth to the eighth day small opacities appear beneath the epithelium but do not involve this cell layer. For this reason the opacities are not stained by fluorescein. The lesions neither ulcerate Bowman's membrane nor do they become vascularized.

EXPERIMENTAL INFECTION, HOST RANGE

Man is the natural host for most types of adenoviruses, and extensive attempts to produce disease in a wide range of common laboratory animals have failed. There is suggestive evidence that infection can be induced in chimpanzees and Syrian hamsters (*Cricetus auratus*) by intranasal inoculation, but serial transmission has not been accomplished, nor have lesions been produced in either host. In these animals, the criterion for infection was

formation of type-specific antibodies following intranasal inoculation of a small dose of virus. The animals to which adenovirus infection could not be transmitted were: infant and adult mice, and infant hamsters inoculated by intranasal, intraperitoneal, intracerebral and intravenous routes, adult guinea pigs inoculated intraperitoneally and intranasally as well as on the cornea and in the anterior chamber of the eye, adult cotton rats by intracerebral and intranasal routes, on the conjunctiva and in the cornea, white rats inoculated intracerebrally and intranasally, rhesus monkeys inoculated intracerebrally, intranasally and subcutaneously, kittens inoculated intracerebrally, adult ferrets inoculated by the intracerebral, the intraperitoneal and the intranasal routes, and embryonated chicken eggs inoculated into the yolk, the allantoic and the amniotic sacs. However, tissue culture technics offer a large number of cell lines which support multiplication of adenoviruses and respond to infection with characteristic cytopathic changes. HeLa cells, epithelial-like cells derived by Gey from an epidermoid carcinoma of the cervix, have proved to be most generally useful for propagation of adenoviruses. In addition, these agents multiply and induce cytopathic changes in a variety of other epithelial-like cell lines derived from several types of cancer, human conjunctival membrane, nasal mucosa, bone marrow, tonsils, adenoids, liver and intestine, and maintained in continuous culture. Adenoviruses will also multiply in tissue cultures prepared from human tracheal epithelium, human amnion, monkey and rabbit kidney and rabbit tracheal epithelium. Undoubtedly, many other susceptible cells have been employed, or will be found to support multiplication of these agents. Although epithelial cells appear to be most susceptible to infection, fibroblasts in mixed cultures also are affected eventually. Whether adenoviruses propagate in these cells or damage the fibroblasts by a toxic action has not been determined.

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ETIOLOGY

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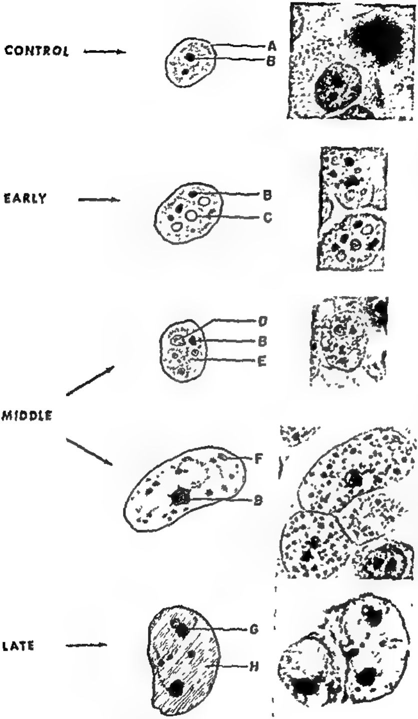

FIG 105. Progressive alterations in nuclei of HeLa cells infected with type 1 adenovirus A diagrammatic representation and a corresponding photograph of typical control and infected cells are shown: (A) nuclear membrane; (B) nucleolus; (C) eosinophilic body; (D) basophilic core of eosinophilic inclusion; (E) rearranged chromatin; (F) basophilic granular cluster; (G) dense basophilic mass; and (H) glassy background Cells were fixed in 95 per cent alcohol and stained with hemotoxylin and eosin (×800-900) (Adapted from Figs 1-10 *in* Boyer, G S, *et al*, 1957, Cytological and cytochemical studies of HeLa cells infected with adenoviruses, J Exper. Med *105*, 195-216)

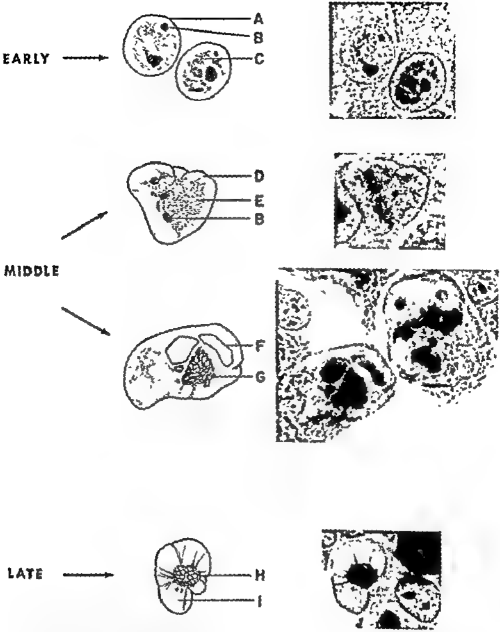

FIG 106 Development of changes in nuclei of HeLa cells infected with type 3 adenovirus A diagrammatic illustration and a corresponding photograph of typical infected cells are presented (A) nuclear membrane, (B) nucleolus, (C) rearranged chromatin, (D) rarefied zone, (E) regularly granular central mass, (F) eosinophilic (initially) or basophilic crystal, (G) coarse network-type central mass, (H) honeycomb-type central mass, (I) compartment of rosette form Cells were fixed in 95 per cent alcohol and stained with hemotoxylin and eosin (×900) (Adapted from Fig 2 and Figs 11-18 *in* Boyer, G S, et al, 1957, Cytological and cytochemical studies of HeLa cells infected with adenoviruses, *J Exper Med* *105*, 195-216)

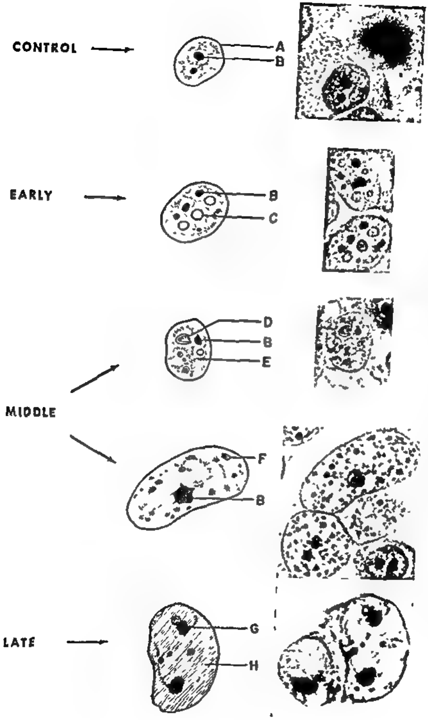

FIG 105 Progressive alterations in nuclei of HeLa cells infected with type 1 adenovirus. A diagrammatic representation and a corresponding photograph of typical control and infected cells are shown (A) nuclear membrane, (B) nucleolus, (C) eosinophilic body, (D) basophilic core of eosinophilic inclusion; (E) rearranged chromatin; (F) basophilic granular cluster; (G) dense basophilic mass, and (H) glassy background. Cells were fixed in 95 per cent alcohol and stained with hemotoxylin and eosin (×800-900) (Adapted from Figs 1-10 *in* Boyer, G S, et al, 1957, Cytological and cytochemical studies of HeLa cells infected with adenoviruses, J Exper. Med *105*, 195-216)

plete within 15 to 30 minutes at room temperature (Ginsberg, 1956b) Cow, horse, or calf serum may neutralize adenoviruses in a manner similar to that of specific neutralizing antibodies (Gold and Ginsberg, 1957). This could be due to the presence of either nonspecific inhibitors or neutralizing antibodies in such animal sera No heat-labile inhibitor in human or animal sera is capable of inactivating adenoviruses

Adenoviruses, unlike a number of other viruses, do not cause hemagglutination of animal erythrocytes under the experimental conditions which have been tested However, when sheep or chicken red blood cells are treated with tannic acid, one or more adenovirus antigens adhere to the cell surface Such cells are then agglutinated by antiserum This test resembles the complement-fixation reaction and is not type-specific (Friedman and Bennett, 1957). The test can be made more nearly type-specific if antiserum is adsorbed with a pool of viruses other than the type for which antibodies are to be measured While viral particles will combine with tanned erythrocytes, the major antigen involved in this reaction is considerably smaller than the viral particle and is easily separated from it by high-speed centrifugation (Ross and Ginsberg, 1958)

Since the original isolation of adenoviruses from tonsils and adenoids and from army recruits with acute respiratory disease (ARD), the relationship of these agents to the etiology of disease has been a foremost problem It is apparent that the viruses can reside in cells of lymphoid tissue, probably for long periods, without causation of overt disease This phenomenon explains their isolation from tonsils, adenoids and mesenteric lymph nodes Whether the adenoviruses which can remain in the quiescent state, particularly types 1, 2, 5 and 6, ever produce recurrent disease is not clear Many strains of adenoviruses have been isolated from cases of clinical illness, and the patients from whom the viruses were isolated developed neutralizing and complement-fixing antibodies In particular, adenoviruses have been implicated etiologically in cases of nonbacterial pharyngitis, pharyngoconjunctival fever, conjunctivitis, ARD, viral or atypical pneumonia not associated with cold hemagglutinins or streptococcus MG agglutinins, and epidemic keratoconjunctivitis Specific types have been associated with certain of the diseases mentioned both in epidemics and in sporadic cases In these instances, the virus has been isolated from secretions of the organ involved, whether the throat, the eye, or the lung Adenovirus has been obtained frequently from stools of ill persons, although gastro-intestinal signs or symptoms are not a feature of any of the diseases with which the adenoviruses have been associated It is considered probable that the virus in pharyngeal secretions is swallowed, and because of its marked stability remains infectious until eliminated in the feces

DIAGNOSIS

The frequency of acute respiratory infections and the relative infrequency with which adenoviruses appear to cause these diseases suggest the difficulties which might be encountered in an attempt to make an etiologic diagnosis on clinical grounds During the course of an epidemic, particularly in recruits in the Armed Forces, it is relatively easy to diagnose either acute respiratory disease (ARD) or nonbacterial pharyngitis However, present evidence indicates that agents other than adenoviruses may also be the cause of nonbacterial pharyngitis and possibly acute respiratory disease (ARD) To some extent the type of virus implicated in a clinical illness may be suspected on clinical grounds Types 4 and 7 viruses are most frequently responsible for epidemics of acute respiratory disease (ARD), but occasional sporadic cases may be difficult to distinguish from infections due to type 3 virus The predominance of symptoms and signs referable to the pharynx in type 3 infections may be helpful in distinguishing nonbacterial pharyngitis from acute respiratory disease (ARD) With both nonbacterial pharyngitis and acute respiratory disease (ARD) constitutional symptoms are usually prominent, so that these symptoms do not assist in the differentiation from influenza and similar infections whose etiology is unknown. Nonbacterial pharyngitis and acute respiratory disease (ARD) must especially be distinguished from undifferentiated acute respiratory diseases of unknown etiology, streptococcal pharyngitis, herpan-

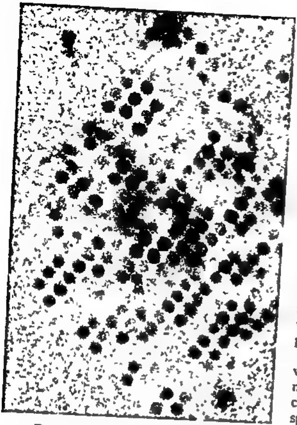

FIG 107 Thin section through an intranuclear crystal composed of type 4 adenovirus. Many of the particles exhibit well-defined internal structure Center-to-center spacing averages 65 mu (×80,000) (Morgan, C, et al., 1956, Structure and development of viruses observed in the electron microscope. IV. Viruses of the RI-APC group J Biophys. & Biochem Cytol. *2*, 351-360)

mains Electronmicroscopic examination of thin sections of types 3, 4 and 7 adenovirus-infected cells indicates that the crystal-like bodies are composed of viral-like particles as illustrated in Figure 107 (Morgan et al, 1956; Harford et al, 1956).

The mechanism by which adenoviruses induce their cytopathic effects is not known. However, these agents can damage susceptible cells in tissue cultures by a seemingly toxic effect in the absence of viral multiplication. Indeed, even virus made noninfectious by heating for 10 minutes at 56° C. can produce this cell damage ...

to the effect of viral infection (Boyer et al, 1957a).

Each adenovirus apparently contains at least 2 antigens which contribute to the immunologic properties and the identification of the agent. The group-specific antigen, by which the numerous types are classified collectively into a single family, is considered to be smaller than the viral particle per se and can be separated from the viral particle by high-speed centrifugation (Hilleman et al, 1955a) The group antigen is most readily detected and quantitated by complement-fixation technics. The reactivity of the viral particle itself in the complement-fixation system has not been determined. Pereira (1956) has shown some type-specificity by complement-fixation titrations using as antigenic material homogenized infected tissue cultures which must contain viral as well as smaller particles

The type-specific antigen is a portion of the viral particle and is readily identified and measured by neutralization technics in tissue culture. Antibody directed against the type-specific antigen neutralizes the infectivity of the homologous virus but does not prevent infection by heterologous types. Although the neutralization reaction does not follow multiple proportions, the relationship between the amount of antibody necessary to neutralize varying quantities of virus is linear on a logarithmic scale and has an exponential form (Ginsberg, 1956b). The slope of the neutralization line is approximately 2 0 for viral types 1, 2, 5 and 6 but is 1 0 for types 3, 4 and 7 viruses (Denny et al, 1958). The differences between types 1, 2, 5 and 6 on the one hand and types 3, 4 and 7 viruses on the other are emphasized again by these quantitative variations in the neutralization reaction (Table 26) Thus, with type 3, 4 or 7 virus a 10-fold change in the quantity of virus employed in the test results in a 10-fold inverse alteration of the neutralization titer obtained. A 10-fold increase in the amount of type 1, 2, 5 or 6 virus mixed with serum causes a 3-fold decrease in the neutralization titer measured. These data indicate the great importance of quantitative factors in neutralization ...

drugs Benefit has been reported from the local use of steroid compounds in epidemic keratoconjunctivitis, but the available data are still insufficient to evaluate this form of therapy It is perhaps not surprising that clinical trials of various specific agents, such as antibiotics, have not shown appreciable benefit to the patients, since Huebner et al (1954) have shown that the adenoviruses are resistant to these agents in the laboratory

EPIDEMIOLOGY

The occurrence of adenovirus infections is probably world-wide, although most of the data thus far available have come from studies in North America and Europe Man appears to be the principal reservoir for those types causing human disease Strains falling into the adenovirus group have been isolated from monkeys and chimpanzees

The importance of the adenoviruses in the total picture of respiratory disease seems at the present time to vary greatly with different population groups or units. In civilian populations, for example, surveys using the presence of complement-fixing and neutralizing antibodies as an index of prior infection indicate widespread infection of children, predominantly with types 1, 2, 5 and 6, and somewhat less extensively with type 3 (Huebner et al, 1954, Hilleman et al, 1955c, Jordan et al, 1956) Only rarely have antibodies for types 4 and 7 been found in children, data for the other types are not available A relatively high proportion of adults have antibodies, indicating previous infection with one or more types of adenoviruses In general, the proportion of persons showing specific antibodies for one or more types increases with age (Fig 108) It is not known how frequently infection with one of these viruses leading to the development of type-

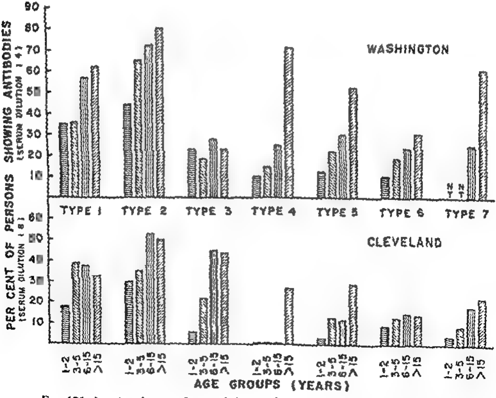

FIG 108 Age distribution of neutralizing antibodies to adenoviruses in Washington and Cleveland (Modified from Jordan, 1957)

gina, influenza, primary atypical pneumonia, psittacosis and Q fever.

Pharyngoconjunctival fever, characterized by the triad of clinical manifestations—fever, pharyngitis and conjunctivitis—occurs usually in epidemic form, is frequently associated with swimming and is caused by type 3 adenovirus in most proved instances, although in one epidemic type 7 virus was shown to be the responsible agent (Ormsby et al, 1957) This syndrome is readily recognized clinically, but again, agents other than adenoviruses also may be etiologically responsible.

Follicular conjunctivitis in epidemics or in sporadic cases has been shown to be due to various types of adenovirus, although type 3 virus has been implicated most frequently Numerous cases of nonbacterial conjunctivitis, including those seen in epidemics, appear to be caused by viruses other than adenoviruses For clinical diagnosis differentiation must be made from bacterial causes of conjunctivitis, inclusion conjunctivitis, Beal's acute follicular conjunctivitis, chronic follicular conjunctivitis (Oxenfeld type), Reiter's disease, conjunctivitis of the erythema multiforme syndrome, and conjunctivitis due to Newcastle disease virus. In addition, differentiation from infections associated with corneal and uveal involvement must be made

Diagnosis of epidemic keratoconjunctivitis is now becoming clearer and differentiation from other forms of keratitis more easily accomplished since the identification of the probable etiologic agent Present evidence indicates that most, if not all, cases of epidemic keratoconjunctivitis are caused by an adenovirus. More specifically, type 8 virus appears to be the cause of the majority of cases, although in some epidemics type 3 or type 7 adenovirus may be the responsible agent The characteristic features, which consist of constitutional manifestations, inflammation of the conjunctiva with edema of the lids, regional lymphadenopathy and superficial corneal opacities, strongly suggest the diagnosis on clinical grounds Epidemiologic characteristics of the disease frequently assist in diagnosis of the infection Differential diagnosis should include trachoma, pemphigus and keratoconjunctivitis or keratitis due to viruses causing such diseases as herpes simplex, lymphogranuloma venereum, variola, vaccinia, measles, mumps, herpes zoster, varicella, molluscum contagiosum and common warts

Laboratory confirmation is required in most cases to establish a definite etiologic diagnosis. The virus can be isolated from pharyngeal, lower respiratory or ocular secretions, or from stools. Secretions from the pharynx or the eye can be obtained by swabbing the organ or washing with broth or balanced salt solution The virus is isolated by inoculating the infected material into a tissue culture of susceptible cells such as HeLa, KB, or human amnion cells. The presence of virus is recognized by the characteristic cytopathic changes which occur in the cells from 2 to 21 days later, depending upon the quantity of virus present in the material obtained from the patient Then the virus can be identified as an adenovirus by the complement-fixation reaction. To determine the type of the virus, neutralization titrations must be done with type-specific sera from immunized rabbits, hamsters or guinea pigs

A serologic diagnosis of adenovirus infection can be accomplished with a serum obtained during the acute stage of the illness (first 5 days), and a second serum procured no less than 14 and preferably 21 days after onset of the disease Complement-fixation titrations with the paired sera and any type of adenovirus as antigen will show an increase in titer of antibody in the second serum specimen To determine the specific virus type which caused the infection, neutralization titrations must be done with the common types of adenoviruses known to cause infection The hemagglutination reaction can be employed for serologic diagnosis but does not appear to offer any advantages over complement-fixation titrations.

TREATMENT

Symptomatic and supportive care, as in other infectious diseases, is the basis of treatment The sulfonamide drugs, penicillin and the so-called broad-spectrum antibiotics have no apparent effect on the course of illness Convalescent serum, gamma globulin and virus vaccine have no value in therapy. Bacterial complications are so rare that there is no need for the prophylactic use of antibiotic

Commission on Acute Respiratory Diseases (1947b, c) which showed that volunteers inoculated with throat washings from a case of acute respiratory disease (ARD) were subsequently immune to reinoculation with the same material

The relationship of adenoviruses to acute respiratory disease (ARD) of recruits was first demonstrated by Hilleman and Werner (1954) who isolated type 4 virus from cases occurring during an epidemic among the troops at Ft Leonard Wood, Mo The particular epidemic was made up of cases of both influenza A and acute respiratory disease (ARD), but after isolation of the viruses the two diseases could be distinguished by appropriate serologic studies (Hilleman et al, 1955b) Subsequent investigations have shown that most cases in outbreaks of acute respiratory disease (ARD) are due to types 4 and 7 viruses, although a few cases may be due to type 3, and one outbreak due to type 14 has been reported (Hilleman et al, 1955d, 1957a, Berge et al, 1955, Dingle, 1955, Woolridge et al, 1956, Rowe et al, 1956a, van der Veen and Kok, 1957) Infection is apparently widespread during the period of the epidemic, since as many as 80 per cent of the recruits have been shown to acquire serologic evidence of infection during an 8-week period encompassing the epidemic Some 20 to 40 per cent of the recruits may require hospitalization, and another 20 per cent may require dispensary care The remainder of the infections are apparently subclinical Duration of infectivity in the individual patient is apparently short, since Rowe et al (1956a) found that virus could be isolated from 88 per cent of patients on the first day of illness and 75 per cent on the second to the fourth day, but that it could not be detected thereafter Duration of specific immunity has not yet been adequately determined, although Hilleman et al (1955d) have demonstrated the persistence of complement-fixing and neutralizing antibodies for 1 year or more, and Jordan et al (1956) have shown the persistence of neutralizing antibodies to type 4 for at least 8 years in civilian adults Katz et al (1957) have also demonstrated the persistence of specific antibodies for periods of at least a year after infection

With the exception of 2 outbreaks of illness due to type 7 virus, 1 in England and 1 in Canada (Tyrrell et al, 1956, Ormsby et al, 1957), outbreaks due to types 4 and 7 have not been reported in civilian populations In fact, only 3 strains of type 4 virus have been isolated from civilian groups Moreover, antibody studies suggest that illnesses due to types 4 and 7 have been extremely rare both in Washington and Cleveland (Fig 108) These findings may explain the epidemic occurrence of acute respiratory disease (ARD) in recruits, since few of them at the time of induction into the services have antibodies to the predominant types, namely, 4 and 7, which cause the disease. Presumably, then, a highly susceptible population is brought together in the barracks of the training camp under conditions favorable to the spread of the virus The mode of spread is undefined but is considered to be via the respiratory tract and to some extent at least may depend upon intimacy of contact

Pharyngitis or Pharyngoconjunctival Fever

Adenovirus infections causing pharyngitis or pharyngoconjunctival fever occur sporadically in both military and civilian populations The epidemic occurrence of this form of adenovirus infection in civilians is found chiefly in children during the summer months (Parrott et al, 1954, Bell et al, 1955, Ginsberg et al, 1955b, Ormsby et al, 1957) The outbreaks are due principally to type 3, two outbreaks due to type 7 have been reported Sporadic cases are most frequently due to type 3, less often to types 2 and 5, and rarely to other types In familial or institutional outbreaks, the virus usually can be isolated only from patients who also show an increase in titers of antibody in their convalescent sera The agent has not been found in well persons regardless of intimacy of contact There appears to be a good correlation between the presence of specific neutralizing antibody and resistance to infection

The attack rate is variable in different outbreaks but may be as high as 70 per cent over a few weeks' time in children in camp (Bell et al, 1955) The incubation period is estimated to be 6 days and the period of communicability to cover a period of an additional 10 days, as indicated by isolation of virus from the pharynx In outbreaks in families the infectiousness is indicated by a secondary attack rate of about 50 per cent in children aged <1 through 9 years, with the rate decreasing with increasing age No sex difference is found in children through 9 years of age, but in the older age groups males appear to be attacked less frequently than fe-

TABLE 27 FREQUENCY OF ADENOVIRUS INFECTION AMONG PERSONS WITH RESPIRATORY ILLNESSES

Location	Population	Diagnostic Method	Number of Cases	Adenovirus Infections No	Adenovirus Infections Per Cent
Ann Arbor (Minuse and Davenport, 1958)	College students	Complement-fixation	814	24	2.9
Chicago (Jackson, 1958)	Adults	" "	124	3	2.4
England (Tyrrell et al, 1956)	Hospital patients	" "	66	2	3.0
	Students and nurses	" "	40	0	—
Glasgow (Grist and Sommerville, 1957)	Adults and children	" "	205	1	0.5
Madison (Evans, 1957)	College students	" "	225	6	2.7
		Virus isolation	290	5	1.7
Cleveland (Jordan et al, 1956)	Families	" "	706	15	2.1
Chicago (Grayston, 1958)	Adults and children	" "	1167	11	0.9
Total			3637	67	1.8

Modified from Jordan (1958)

specific antibodies is associated with symptoms of respiratory disease Attempts to isolate the agents from patients with respiratory disease in civilian groups, such as hospitalized patients, students, nurses, families, etc, suggest that these viruses cause a very small proportion of the cases of respiratory disease The frequency of isolation of all types has varied from less than 1 per cent to approximately 2 per cent of all respiratory illnesses in various series Complement-fixation and neutralizing antibody studies in the same group of patients indicate that at the most only 4 to 5 per cent of the respiratory illnesses are due to adenoviruses (Table 27).

In addition to sporadic occurrence, however, epidemics of adenovirus infections occur in military recruits and in civilian populations, especially in children, so that further discussion of the epidemiology of the adenovirus group will consider acute respiratory disease (ARD), nonbacterial pharyngitis and epidemic keratoconjunctivitis separately.

ACUTE RESPIRATORY DISEASE (ARD)

The occurrence of epidemics of acute respiratory disease (ARD) or febrile catarrh in [illegible]

beginning 2 or 3 weeks after the arrival of the men on the post and running its course in the subsequent 3 to 4 weeks In this respect its behavior is similar to that of influenza Epidemics occur particularly during the winter months During this season the attack rate may be high, and from 20 to 40 per cent of the recruits may be sufficiently ill to require hospitalization In training posts where there is a continuous induction of recruits at weekly or monthly intervals, each unit in succession will experience the epidemic wave with remarkable consistency During summer months the epidemic occurrence of the disease may disappear entirely, but those units arriving at the post during the summer may undergo an epidemic wave in the fall even after several weeks of training. Men who comprise the cadre responsible for training the recruits and have had months or years of prior military experience seldom are involved in these outbreaks The epidemic wave in each organizational unit appears to confer group immunity—a process known as seasoning—since this unit will not again undergo an epidemic of acute respiratory disease (ARD). In this respect the disease differs from influenza, since outbreaks of influenza involve both recruits and seasoned men Thus the epidemiologic behavior suggests that there is a high degree of susceptibility among recruits, that the outbreaks are caused by a single agent or by closely related agents and that the disease is followed by relatively solid immunity. The latter observation was confirmed by the volunteer studies of the

Commission on Acute Respiratory Diseases (1947b, c) which showed that volunteers inoculated with throat washings from a case of acute respiratory disease (ARD) were subsequently immune to reinoculation with the same material

The relationship of adenoviruses to acute respiratory disease (ARD) of recruits was first demonstrated by Hilleman and Werner (1954) who isolated type 4 virus from cases occurring during an epidemic among the troops at Ft Leonard Wood, Mo The particular epidemic was made up of cases of both influenza A and acute respiratory disease (ARD), but after isolation of the viruses the two diseases could be distinguished by appropriate serologic studies (Hilleman et al, 1955b) Subsequent investigations have shown that most cases in outbreaks of acute respiratory disease (ARD) are due to types 4 and 7 viruses, although a few cases may be due to type 3, and one outbreak due to type 14 has been reported (Hilleman et al, 1955d, 1957a, Berge et al, 1955, Dingle, 1955, Woolridge et al, 1956, Rowe et al, 1956a, van der Veen and Kok, 1957) Infection is apparently widespread during the period of the epidemic, since as many as 80 per cent of the recruits have been shown to acquire serologic evidence of infection during an 8-week period encompassing the epidemic Some 20 to 40 per cent of the recruits may require hospitalization, and another 20 per cent may require dispensary care The remainder of the infections are apparently subclinical Duration of infectivity in the individual patient is apparently short, since Rowe et al. (1956a) found that virus could be isolated from 88 per cent of patients on the first day of illness and 75 per cent on the second to the fourth day, but that it could not be detected thereafter Duration of specific immunity has not yet been adequately determined, although Hilleman et al (1955d) have demonstrated the persistence of complement-fixing and neutralizing antibodies for 1 year or more, and Jordan et al (1956) have shown the persistence of neutralizing antibodies to type 4 for at least 8 years in civilian adults Katz et al (1957) have also demonstrated the persistence of specific antibodies for periods of at least a year after infection

With the exception of 2 outbreaks of illness due to type 7 virus, 1 in England and 1 in Canada (Tyrrell et al, 1956, Ormsby et al, 1957), outbreaks due to types 4 and 7 have not been reported in civilian populations In fact, only 3 strains of type 4 virus have been isolated from civilian groups Moreover, antibody studies suggest that illnesses due to types 4 and 7 have been extremely rare both in Washington and Cleveland (Fig 108) These findings may explain the epidemic occurrence of acute respiratory disease (ARD) in recruits, since few of them at the time of induction into the services have antibodies to the predominant types, namely, 4 and 7, which cause the disease. Presumably, then, a highly susceptible population is brought together in the barracks of the training camp under conditions favorable to the spread of the virus The mode of spread is undefined but is considered to be via the respiratory tract and to some extent at least may depend upon intimacy of contact

Pharyngitis or Pharyngoconjunctival Fever

Adenovirus infections causing pharyngitis or pharyngoconjunctival fever occur sporadically in both military and civilian populations The epidemic occurrence of this form of adenovirus infection in civilians is found chiefly in children during the summer months (Parrott et al, 1954, Bell et al, 1955, Ginsberg et al, 1955b, Ormsby et al, 1957) The outbreaks are due principally to type 3, two outbreaks due to type 7 have been reported Sporadic cases are most frequently due to type 3, less often to types 2 and 5, and rarely to other types In familial or institutional outbreaks, the virus usually can be isolated only from patients who also show an increase in titers of antibody in their convalescent sera The agent has not been found in well persons regardless of intimacy of contact There appears to be a good correlation between the presence of specific neutralizing antibody and resistance to infection

The attack rate is variable in different outbreaks but may be as high as 70 per cent over a few weeks' time in children in camp (Bell et al, 1955) The incubation period is estimated to be 6 days and the period of communicability to cover a period of an additional 10 days, as indicated by isolation of virus from the pharynx In outbreaks in families the infectiousness is indicated by a secondary attack rate of about 50 per cent in children aged <1 through 9 years, with the rate decreasing with increasing age No sex difference is found in children through 9 years of age, but in the older age groups males appear to be attacked less frequently than fe-

males, the difference being in the order of 1.2 in Bell's studies

The mode of spread is generally considered to be person-to-person via the respiratory tract, but the virus has been isolated from stools, and there is some indication that certain outbreaks have been related to swimming pools (Bell et al, 1955; Ormsby and Aitchison, 1955, Cockburn et al., 1956). This observation has led to the hypothesis that the eye might be the portal of entry of infection Some support for this concept is obtained from the volunteer studies of Bell et al. (1956b) who were able to induce conjunctivitis with or without nasopharyngitis by rubbing the virus on the palpebral conjunctiva with a swab but obtained no illnesses, or only minor illnesses, when the virus was inoculated intranasally In contrast with these results, however, Roden et al (1956) were able to transmit infections to volunteers by the intranasal inoculation of type 1 adenovirus.

The epidemiologic significance of the isolation of various types of adenoviruses from tonsillar and adenoidal tissue removed surgically has not yet been determined It is possible that these viruses lead a symbiotic or latent existence in lymphoidal tissue and that with the proper stimulus they may excite recurrent infection in the person harboring them, or they may spread to initiate other cases or an epidemic In a few instances the same strain of virus has been isolated from the pharynx of an individual at times of recurrent respiratory infections Whether or not the same virus was the cause of these infections has not been determined

The relationship between the properties of the virus in laboratory cultures and the epidemiologic behavior is not clear In some situations (Dingle et al, 1955), for example, the type 3 virus does not seem to spread widely in the population during an epidemic period In general, the virus can be recovered only from persons who were ill, those who remained well give no serologic evidence of infection. Subclinical infection, which occurs readily with many viral diseases, such as poliomyelitis, thus does not seem to be common for the type 3 virus This observation has led to the postulate that the epidemiologic behavior of the virus may be related in part to the fact that it is neither adsorbed well to tissue cells nor easily released However, the epidemiologic behavior of acute respiratory disease (ARD) due to types 4 and 7 adenoviruses is in marked contrast, as indicated in the preceding section, since in these outbreaks the viruses appear to spread widely and rapidly and the rate of subclinical infection is high, as shown by serologic studies Yet the behavior of these types in the laboratory with respect to adsorption to and release from cells in tissue culture is comparable with that of other types.

Conjunctivitis and Keratoconjunctivitis

There is insufficient information to describe the epidemiologic behavior of follicular conjunctivitis occurring apart from associated infections of the respiratory tract. Presumably, however, its behavior would be similar to that of pharyngitis and pharyngoconjunctival fever described above. The volunteer studies of Bell et al (1956b) have demonstrated that resistance and susceptibility to infection induced by swabbing the virus onto the conjunctiva can be correlated with the presence or the absence, respectively, of specific antibody in the sera.

Sporadic cases of keratoconjunctivitis occur not infrequently and in some instances have been associated with types 3, 7 and 8 adenoviruses by isolation of the virus from scrapings of the conjunctiva and by the demonstration of increases in antibody titers to the virus in convalescent sera However, the epidemic occurrence of the disease is more striking, and large outbreaks have occurred in the Far East, in Hawaii, in the United

toconjunctivitis show a significant increase in neutralizing antibodies to adenovirus type 8 during the acute phase of the disease and a gradual loss of titer thereafter Moreover, the sera from patients with epidemic keratoconjunctivitis in Japan, Italy, Switzerland and North America have been shown to contain neutralizing antibodies to this virus, whereas such antibodies were absent in the general population in these areas Thus the present evidence suggests that adenovirus type 8 is involved in the production of the disease, but further data are needed to establish a firm etiologic relationship The mode of spread of epidemic keratoconjunctivitis is not known, although trauma to the conjunctiva from dust and dirt in offices and dispensaries where

proper aseptic technics are not employed may be a factor in its distribution

CONTROL MEASURES

The general measures that have been employed in an attempt to control the spread of adenovirus infections are similar to those that have been employed for other diseases presumably spread via the respiratory tract. The principal studies have been carried out in military populations composed of recruits and have consisted of such procedures as the oiling of floors and blankets, the use of double bunks to increase the space between beds, ultraviolet lights and the use of aerosols. These various procedures have had either little or no effect, or at best have resulted in a reduction of only approximately 5 per cent. In general, isolation procedures for patients have little or no effect. Chlorination of swimming pools is advisable, should it prove that adenovirus infections may be spread from such a source, since Clarke et al (1956) have shown that these agents are sensitive to the action of chlorine.

With respect to epidemic keratoconjunctivitis, rigid asepsis in the medical divisions of factories and plants should be employed to prevent the spread of infection by hands of attendants or by instruments. Isolation of patients with the disease has also been advised.

The use of specific preventive measures such as gamma globulin or adenovirus vaccines is indicated under special circumstances in which attack rates can be predicted to be high, such as at military recruit training centers. That a vaccine, when obtained, would be effective for the prevention of acute respiratory disease (ARD) of recruits was suggested by the epidemiologic behavior of the disease, which indicated the development of a relatively permanent immunity in a population that had experienced an epidemic, and by the demonstration of immunity in the individual person by challenge inoculation in volunteer studies. In addition, (1) susceptibility or resistance to clinical infection was correlated directly with the absence or the presence, respectively, of circulating neutralizing antibodies for type 4 virus, (2) such antibodies developed during convalescence (Ginsberg et al, 1955a), and (3) the adenoviruses appear to be good antigens. On theoretical grounds, therefore, it could be predicted that active immunization and possibly passive immunization would be effective for the prevention of ARD in recruits (Dingle, 1955). The studies of Huebner et al (1955), Bell et al (1956a), Hilleman et al (1956, 1957c, d) and Stallones et al (1957) have demonstrated that a satisfactory vaccine can be prepared containing 2 or 3 types of adenoviruses (types 3, 4 and 7). The vaccines are antigenic, and 1 dose apparently gives almost maximum antibody response. Their use in several studies has demonstrated a reduction of from 85 to 95 per cent in the attack rate of adenovirus infections in immunized groups in comparison with control groups not receiving such vaccine.

There is little reason to doubt that adenovirus vaccines can produce antibodies in children and that resistance to infection would follow their use in civilian populations. At the present time the low incidence of adenovirus infections in the civilian population, as already pointed out, would raise serious question as to the advisability of the widespread use of an adenovirus vaccine except for special situations, such as in orphanages, where a high incidence of infection might be predicted. In fact, Jordan (1958) has estimated, on the basis of data on incidence of adenovirus infections in civilian populations, that the use of a vaccine which completely prevented infection would result in only a 6 per cent reduction in the number of common respiratory illnesses experienced by an average child during the first 10 years of life. Similarly, the low incidence of infection in other civilian groups, such as university student populations, does not warrant immunization with adenovirus vaccine at the present time.

No data are yet available regarding the possible efficacy of a type 8 adenovirus vaccine for the prevention of epidemic keratoconjunctivitis. However, the relatively rapid fall of specific antibodies in the sera of patients convalescent from the disease suggests the possibility that any immunity conferred by such a vaccine might be of relatively short duration and that repeated inoculations of the vaccine might be necessary to provide effective protection.

REFERENCES

Andrews, B E, McDonald, J. C, Thorburn, W B, and Wilson, J S, 1956, Respiratory virus infections in R A F, 1954-55, with particular reference to influenza and A P C viruses, Brit M J *1*, *1203-1207*

Aoki, H, and Kasahara, C, 1941, Epidemic keratoconjunctivitis, Acta Soc Ophth Japan *45*, *573*

Barski, G, 1956, Character of the specific cellular lesion caused *in vitro* by the viruses of the APC group and its diagnostic value, Ann Inst Pasteur *91*, 614-622

Bell, J A, Hantover, M J, Huebner, R J, and Looslı, C. G, 1956a, Efficacy of trivalent adenovirus (APC) vaccine in naval recruits Progress report J A M A *161*, 1521-1525

Bell, J A, Rowe, W P, Engler, J I, Parrott, R H, and Huebner, R J, 1955, Pharyngoconjunctival fever Epidemiological studies of a recently recognized disease entity J A M A *157*, 1083-1092

Bell, J A, Ward, T G, Huebner, R J, Rowe, W P, Suskind, G, and Paffenbarger, R S, 1956b, Studies of adenoviruses (APC) in volunteers, Am J Pub Health *46*, 1130-1146

Berge, T O, England, B, Mauris, C, Shuey, H E, and Lennette, E H, 1955, Etiology of acute respiratory disease among service personnel at Fort Ord, California, Am J Hyg *62*, 283-294

Bietti, G B, and Bruna, F, 1957, Epidemic keratoconjunctivitis in Italy Some contributions to its clinical aspects, epidemiology and etiology Am J Ophth *43*, 50-57

Boyer, G S, Denny, F W, Jr, and Ginsberg, H S, 1957b, Unpublished data

Boyer, G S, Leuchtenberger, C, and Ginsberg, H S, 1957a, Cytological and cytochemical studies of HeLa cells infected with adenoviruses, J Exper Med *105*, 192-216

Chany, C, Lépine, P, Lelong, M, Le Tan Vinh, Satgé, P and Virat, J, 1958, Severe and fatal pneumonia in infants and young children associated with adenovirus infection, Am J Hyg *67*, 367-378

Cheever, F S, 1951, A possible relationship between viruses of St Louis encephalitis and epidemic keratoconjunctivitis, Proc Soc Exper. Biol & Med *77*, 125-129

Clarke, N A, Stevenson, R E, and Kabler, P W, 1956, The inactivation of purified type 3 adenovirus in water by chlorine, Am J Hyg *64*, 314-319

Cockburn, T A, Rowe, W P, and Huebner, R J, 1956, Relationship of the 1951 Greeley, Colorado, outbreak of conjunctivitis and pharyngitis to type 3 APC virus infection, Am. J Hyg *63*, 250-253

Commission on Acute Respiratory Diseases, 1944, Epidemiology of atypical pneumonia and acute respiratory disease at Fort Bragg, North Carolina, Am. J Pub Health *34*, 335-346

———, 1946, Acute respiratory disease among new recruits, Am J. Pub Health *36*, 439-450

———, 1947a, Clinical patterns of undifferentiated and other acute respiratory diseases in army recruits, Medicine *26*, 441-464

———, 1947b, Experimental transmission of minor respiratory illness to human volunteers by filter-passing agents I Demonstration of two types of illness characterized by long and short incubation periods and different clinical features J Clin. Invest *26*, 957-973.

———, 1947c, Experimental transmission of minor respiratory illness to human volunteers by filter-passing agents II. Immunity on reinoculation with agents from the two types of minor respiratory illness and from primary atypical pneumonia J Clin Invest, *26*, 974-982

Dascomb, H E, and Hilleman, M R, 1956, Clinical and laboratory studies in patients with respiratory disease caused by adenoviruses (RI-APC-ARD Agents), Am J Med *21*, 161-174

Denny, F W, Jr, Boyer, G S, and Ginsberg, H S, 1958, Characteristics of adenoviruses types 5, 6 and 7, Fed Proc (abstract) *17*, 509

Dingle, J H, 1955, Respiratory disease research, U S Armed Forces M J *6*, 1249-1264

Dingle, J H, Ginsberg, H S, Jordan, W. S, Jr, Katz, S, and Badger, G F, 1955, Relationship of certain characteristics of the new respiratory viruses to the clinical and epidemiological behavior of non-bacterial pharyngitis, Tr. A Am Physicians *68*, 73-77

Drouhet, V, 1957, Study of an APC virus (adenovirus) isolated from a case of fatal pneumonia, Ann Inst. Pasteur *93*, 138-141

Enders, J F, Bell, J A, Dingle, J H, Francis, T, Jr, Hilleman, M R, Huebner, R J, Payne, A M-M, 1956, Adenoviruses, Group name proposed for new respiratory-tract viruses, Science *124*, 119-120

Evans, A S, 1957, Acute respiratory disease in University of Wisconsin students, New England J Med *256*, 377-384

Fisher, T N, and Ginsberg, H S, 1957, Accumulation of organic acids by HeLa cells infected with type 4 adenovirus, Proc Soc Exper Biol & Med *95*, 47-51

Friedman, M, and Bennett, C R, 1957, A hemagglutination test for detection of adenovirus antibodies, Proc Soc Exper Biol & Med *94*, 712-717

Ginsberg, H S, 1956a, Characteristics of the new respiratory viruses (adenoviruses) II Stability to temperature and pH alterations Proc Soc Exper Biol & Med *93*, 48-52

———, 1956b, Characteristics of the new respiratory viruses (adenoviruses) I Qualitative and quantitative aspects of the neutralization reaction J Immunol *77*, 271-278

———, 1958, Characteristics of adenoviruses III Reproductive cycle of types 1 to 4 J Exper Med *107*, 133-152

———, 1957, Biological and physical properties of the adenoviruses, Ann. New York Acad Sc *67*, 383-391

Ginsberg, H S, Badger, G F, Dingle, J. H, Jordan, W S, Jr, and Katz, S, 1955a, Etiologic relationship of the RI-67 agent to Acute Respiratory Disease (ARD), J Clin. Invest *34*, 820-831.

Ginsberg, H S, Gold, E, Jordan, W S, Jr., Katz, S, Badger, G F, and Dingle, J H, 1955b, Relation of the new respiratory agents to acute respiratory diseases, Am J Pub Health *45*, 915-922

Gold, E, and Ginsberg, H S, 1957, Inhibition of adenoviruses by normal animal sera, Fed Proc (abstract) *16*, 414

Grayston, J T, 1958, quoted in Jordan, W S, Jr., A M A Arch Int Med *101*, 54-59

Grist, N R, and Sommerville, R G, 1957, Respiratory virus infections in hospital and in general practice, 1954-6 Brit M J *1*, 378-380

Harford, C G, Hamlin, A, Parker, E, and van Ravenswaay, T, 1956, Electron microscopy of HeLa cells infected with adenoviruses, J Exper Med *104*, 443-454

Hilleman, M R, Gauld, R L, Butler, R L, Stallones, R A, Hedberg, C L, Warfield, M S, and Anderson, S A, 1957a, Appraisal of occurrence of adenovirus-caused respiratory illness in military populations, Am J Hyg *66*, 29-41

Hilleman, M R, Hodges, R E, Warfield, M S, and Anderson, S A, 1957b, Acute respiratory illness in volunteers following intramuscular administration of live adenovirus, J Clin Invest *36*, 1072-1080

Hilleman, M R, Stallones, R A, Gauld, R L, Warfield, M S, and Anderson, S A, 1956, Prevention of acute respiratory illness in recruits by adenovirus (RI-APC-ARD) vaccine, Proc Soc Exper Biol & Med *92*, 377-383

———, 1957c, Vaccination against acute respiratory illness of adenovirus (RI APC-ARD) etiology, Am J Pub Health *47*, 841-847

Hilleman, M R, Tousimis, A J, and Werner, J H, 1955a, Biophysical characterization of the RI (RI-67) viruses, Proc Soc Exper Biol & Med *89*, 587-593

Hilleman, M R, Warfield, M S, Anderson, S A, and Werner, J H, 1957d, Adenovirus (RI-APC-ARD) vaccine for prevention of acute respiratory illness I Vaccine development J A M A *163*, 4-9

Hilleman, M R, and Werner, J H, 1954, Recovery of new agent from patients with acute respiratory illness, Proc Soc Exper Biol & Med *85*, 183-188

Hilleman, M R, Werner, J H, Adair, C V, and Dreisbach, A R, 1955b, Outbreak of acute respiratory illness caused by RI 67 and influenza A viruses, Fort Leonard Wood, 1952-53 Am J Hyg *61*, 163-173

Hilleman, M R, Werner, J H, Dascomb, H E, and Butler, R L, 1955c, Epidemiologic investigations with respiratory disease virus RI-67, Am J Pub Health *45*, 203-210

Hilleman, M R, Werner, J H, Dascomb, H E, Butler, R L, and Stewart, M T, 1955d, Epidemiology of RI (RI-67) group respiratory virus infections in recruit populations, Am J Hyg *62*, 29 42

Huebner, R J, Bell J A, Rowe, W P, Ward, T G, Suskind, R G, Hartley, J W, and Paffenbarger, R S, 1955, Studies of adenoidal-pharyngeal-conjunctival vaccines in volunteers, J A M A *159*, 986-989

Huebner, R J, and Rowe, W P, 1957, Adenoviruses as etiologic agents in conjunctivitis and keratoconjunctivitis, Am J Ophth *43*, 20-25

Huebner, R J, Rowe, W P, Ward, T G, Parrott, R H, and Bell, J A, 1954, Adenoidal-pharyngeal-conjunctival agents A newly recognized group of common viruses of the respiratory system New England J Med *251*, 1077-1086

Jackson, G, G, 1958, quoted in Jordan, W S, Jr, Arch Int Med *101*, 54-59

Jawetz, E, Hanna, L, Kimura, S J, and Thygeson, P, 1956a, Clinical diseases of adults associated with sporadic infections by APC viruses, A M A Arch Int Med *98*, 71-79

Jawetz, E, Kimura, S J, Hanna, L, Coleman, V R, Thygeson, P, and Nicholas, A, 1955b, Studies on the etiology of epidemic keratoconjunctivitis, Am J Ophth *40*, 200-209

Jawetz, E, Kimura, S, Nicholas, A N, Thygeson, P, and Hanna, L, 1955a, New type of APC virus from epidemic keratoconjunctivitis, Science *122*, 1190-1191

Jawetz, E, Thygeson, P, Hanna, L, Nicholas, A, and Kimura, S, 1956b, Antibodies to APC virus type 8 in epidemic keratoconjunctivitis, Proc Soc Exper Biol & Med *92*, 91-95

———, 1957, The etiology of epidemic keratoconjunctivitis, Am J Ophth *43*, 79-83

Jordan, W S, Jr, 1958, Occurrence of adenovirus infections in civilian populations, A M A Arch Int Med *101*, 54-59

Jordan, W S, Jr, Badger, G F, Curtiss, C, Dingle, J H, Ginsberg, H S, and Gold, E, 1956, A study of illness in a group of Cleveland families X. The occurrence of adenovirus infections Am J Hyg *64*, 336-348

Katz, S, Jordan, W S, Jr, Badger, G F, and Dingle, J H, 1957, Studies of complement-fixing and neutralizing antibodies against certain adenoviruses, J Immunol *78*, 118-121

Kjellen, L, Lagermalm, G, Svedmyr, A M, and Thorsson, K G, 1955, Crystalline-like patterns in the nuclei of cells infected with an animal virus, Nature, London *175*, 505-506

Minuse, E, and Davenport, F M, 1958, quoted in Jordan, W S, Jr, A M A Arch Int Med *101*, 54 59

Mitsui, Y, Hanabusa, J, Minode, R, and Ogata, S, 1957, Effect of inoculating adenovirus (APC virus) type 8 into human volunteers, Am J Ophth *43*, 84-90

Morgan, C, Godman, G C, Rose, H M, Howe, C, and Huang, J S, 1957, Electron microscopic and histochemical studies of an unusual crystalline protein occurring in cells infected by type 5 adenovirus Preliminary observations J Biophys & Biochem Cytol *3*, 505-508

Morgan, C, Howe, C, Rose, H M, and Moore, D H, 1956, Structure and development of viruses observed in the electron microscope IV Viruses of the RI APC group J Biophys & Biochem Cytol *2*, 351-360

Neva, F A, and Enders, J F, 1954, Isolation of a cytopathogenic agent from an infant with a disease

in certain respects resembling *roseola infantum*, J Immunol *72, 315-321.*

Ormsby, H L, and Aitchison, W S, 1955, Pharyngeal-conjunctival fever due to swimming pool contact, Canad M A J *73, 864-866*

Ormsby, H L, Fowle, A M C, and Doane, F, 1957, Canadian cases of adenovirus infection, 1951-56, Am J Ophth *43, 17-25*

Parrott, R H, Rowe, W P, Huebner, R J, Bernton, H. W, and McCullough, N B, 1954, Outbreak of febrile pharyngitis and conjunctivitis associated with type 3 adenoidal-pharyngeal conjunctival virus infection, New England J Med. *251, 1087-1090*

Pereira, H G, 1956, Typing of adenoidal-pharyngeal-conjunctival (APC) viruses by complement-fixation, J Path & Bact *72*, 105-109

Roden, A T, Pereira, H G, and Chapronsere, D M, 1956, Infection of volunteers by a virus (APC type 1) isolated from human adenoid tissue, Lancet *2*, 592-596

Ross, E, and Ginsberg, H S, 1958, Hemagglutination with adenoviruses Nature of viral antigen Proc Soc Exper Biol & Med *98*, 501-505

Rowe, W P, Hartley, J W, and Huebner, R J, 1956b, Additional serotypes of the APC virus group, Proc Soc Exper Biol & Med *91*, 260-262

———, 1958, Serotype composition of the adenovirus group Proc Soc Exper Biol & Med *97*, 465-470

Rowe, W P, Huebner, R J, Gilmore, L K, Parrott, R H, and Ward, T G, 1953, Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture, Proc Soc Exper Biol & Med *84, 570-573*

Rowe, W P, Seal, J R, Huebner, R. J, Whiteside, J E, Woolridge, R L, and Turner, H C, 1956a, A study of the role of adenoviruses in acute respiratory infections in a Navy recruit population, Am J. Hyg *64*, 211-219

Ruchman, I, 1951, Relationship between epidemic keratoconjunctivitis and St Louis encephalitis viruses, Proc Soc. Exper Biol & Med *77, 120-125*

Sanders, M, and Alexander, R C, 1943, Epidemic keratoconjunctivitis I Isolation and identification of a filterable virus J. Exper. Med *77*, 71-96

Southam, C. M, Hilleman, M R, and Werner, J H, 1956, Pathogenicity and oncolytic capacity of *RI virus strain RI-67 in man, J Lab & Clin Med 47, 573-582*

Stallones, R. A, Hilleman, M R, Gauld, R L, Warfield, M S, and Anderson, S, 1957, Adenovirus (RI-APC-ARD) vaccine for prevention of acute respiratory illness 2 Field evaluation JAMA *163, 9-15*

Stuart-Harris, C. H, Andrewes, C H, Smith, W, Chalmers, D K M, Cowen, E G H, and Hughes, D L, 1938, A study of epidemic influenza With special reference to the 1936-37 epidemic Med Res Council London, Special Reports, Series 228.

Tyrrell, D A J, Balducci, D, and Zaiman, T E, 1956, *Acute infections of the respiratory* tract and the adenoviruses, Lancet *2*, 1326-1330

van der Veen, J, and Kok, G, 1957, Isolation and typing of adenoviruses recovered from military recruits with acute respiratory disease in the Netherlands, Am. J Hyg *65*, 119-129

Woolridge, R L, Grayston, J T, Whiteside, J E, Loosli, C G, Friedman, M, and Pierce, W. E, 1956, Studies on acute respiratory illness in Naval recruits *with emphasis on the adenoviruses* (APC-RI), J Infect Dis *99*, 182-206

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31

Influenza

(SYNONYMS *La grippe*, grip, febrile catarrh, catarrhal fever, acute nasopharyngitis, epidemic catarrh, epidemic influenza)

INTRODUCTION

Influenza is an acute infectious respiratory disease of man, commonly encountered in epidemic form, caused by one of the influenza viruses Clinically, there is sudden onset with fever, myalgias, pharyngitis, cough, leukopenia, the course is usually self-limiting in 3 to 4 days Constitutional manifestations ordinarily overshadow respiratory features Complication of bronchitis and bronchopneumonia are frequent Epidemics are characterized by rapid dissemination high morbidity and low mortality, however, they may be *world-wide and of such severity as to attain* catastrophic proportions Four distinct immunologic types of influenza virus A, B, C and D, have been recognized and classified according to the plan of Horsfall et al (1940) Influenza A and influenza B have been encountered most frequently and therefore are the best studied

HISTORY

What is now called influenza is readily recognized in dramatic descriptions covering many centuries of epidemics arising explosively in a population, spreading rapidly and widely over large areas The comment is made repeatedly that many sickened but few died of it except the old and the infirm The clinical manifestations, too, are in keeping with modern observations Short wrote of the epidemic of 1557—"it began with a roughness of the jaws, small cough, then a strong fever, with a pain of the head, back and legs, some felt as though they were over the breast and had a weight at the stomach, all which continued to the third day at the farthest, there the fever went off with a sweat or bleeding at the nose In some few, it turned to pleurisy, or fatal peri pneumony" (Thompson 1852)

From 1510 to 1930 some 30 of the prevalences were clearly considered pandemic, and numerous outbreaks of lesser extent were described For example, between 1800 and 1875 Hirsch (1883) records 55 years in which he states influenza was epidemic in various parts of the world But obviously the more pronounced occurrences have been best described The outbreak of 1743 was considered a virulent pandemic, and Jordan (1927) states that the actual number of deaths occurring in London during the height of the epidemic was as great in proportion to the population as it was in American cities in 1918 *It was in this epidemic that the name* "influenza" was derived from the Italian phrase attributing the origins of the disease to *un influenza di freddo* That of 1782 was called a great pandemic in Asia and Europe with apparently a high frequence of complications At this time there was strong support for the thesis that the disease was infectious and contagious with a specific cause rather than the result of meteorologic or telluric influences However, it was not until the

pandemic period of 1889-92 that relatively accurate data were compiled and bacteriologic studies undertook to seek a specific etiologic agent. Pfeiffer (1892) then described *H. influenzae* and assigned to it primary causative significance And in 1889-90 Abbott demonstrated the independence of direction of spread from that of prevailing winds The entire history of influenza was climaxed by the greatest of all the pandemics in 1918-19 which, it is estimated, resulted in the death of some 20 million persons The extensive observations of that episode and efforts to explain it have since been a dominant concern of students of respiratory disease and epidemiology They have been reviewed exhaustively by Thomson and Thomson (1933, 1934)

Despite the variations, major outbreaks retain close similarity in their apparently abrupt development in an area, their wide dispersion, the mild 3-day nature of the majority of illnesses, the relatively low case fatality, and the tendency to subside rapidly and be followed by lesser waves over the next several years. These trailers have been considered part of the pandemic phenomenon

Modern knowledge of the causal agents of human influenza begins with isolation in ferrets of a virus from cases of influenza by Smith, Andrewes and Laidlaw (1933) who also noted that convalescent sera of the patients contained neutralizing antibodies to the virus The isolation of virus was promptly confirmed in America (Francis, 1934), and it was demonstrated that the specific antibodies actually develop during the disease. Virus was adapted to mice, serologic tests were developed, and opportunity to study the nature and the distribution of the virus was thus provided It was also shown that the virus induced in human volunteers a disease clinically characteristic of influenza This virus is now called Type A, and the disease influenza A has been followed in repeated outbreaks, especially 1936-37, 1943, 1947-49, 1957.

Studies at this time demonstrated that the virus of swine influenza (Shope, 1931) was antigenically related to, but not identical with, influenza A virus from man It was shown (Magill and Francis, 1936, 1938; Francis and Magill, 1938; Smith and Andrewes, 1938) that antigenic differences were demonstrable between strains of Type A, and the subject of antigenic variation among strains of influenza virus has become a problem of great theoretical and practical concern.

In 1940 a second etiologic type, Type B influenza virus, was identified with the epidemic current at that time as well as with earlier prevalences (Francis, 1940, Magill, 1940). It, too, has since been recognized in widespread outbreaks.

A virus isolated from a patient in 1949 (Taylor) was found to be distinct from Types A and B viruses, and the antibody response of the patient was specific for the new virus In 1950 another strain was isolated and shown to be associated with an institutional outbreak of influenza This virus has been designated Type C influenza virus (Francis et al., 1950). Later, several small outbreaks have been identified in various countries, but wide distribution of the infection is shown by the high frequency of antibody in the general population

More recently a virus isolated in Japan from newborn pneumonitis (Sendai virus, hemagglutinating virus of Japan) (Kuroya et al, 1953) was shown by serologic studies to be prevalent in the United States; on this basis and the similarity in characteristics of that virus to other influenza viruses it was tentatively designated Type D (Jensen et al, 1955, Francis, 1955) Scattered cases have been detected, and in 1956 a large outbreak of influenza in Vladivostok was reported from which virus of this type was isolated, and serologic studies indicated its etiologic relationship (Gerngross 1957, Gorbunova et al, 1957)

Variations of strains within the type have been observed in different years The 2 most distinct variants of Type A influenza virus recovered from man have been those of 1946-47 (designated A-prime by the Commission on Influenza) and the Asian strains of 1957 Each has been associated with widespread outbreaks. Modifications of Type B strains have also appeared with those of 1945, 1952 and 1954-55 quite different antigenically from earlier strains. In view of the multiple types and subtypes and their comparative independence of distribution, interpretation of waves and recurrences noted in earlier descriptions of pandemic prevalences is at present uncertain.

CLINICAL PICTURE

The symptoms of uncomplicated influenza are disturbing to no one but the patient unless they affect a sufficient number of persons

to interrupt the functions of the group or the community

The incubation period of influenza is ordinarily 1 to 2 days Experimental infection by direct inhalation of fine sprays of virus has at times exhibited even shorter incubation periods The onset of illness is abrupt with sudden chilliness or distinct chill, fatigue, headache and general achiness The temperature rises rapidly in 12 to 24 hours to *a level of 101° to 104° F* *Diffuse headache* and severe muscular aching of the back and the extremities are usual in adults but are generally less marked in children The patient experiences a sense of fatigue or weakness building rapidly to prostration Ocular tenderness, conjunctival injection and watery eyes are common, usually without photophobia *Although nasal or nasopharyngeal* irritation may be noted early, constitutional symptoms are more prominent than evidence of respiratory infection Coryza is rarely prominent, but sneezing and mild discharge are not uncommon Epistaxis, probably from viral injury to nasal epithelium, occurs in a variable manner Truly sore throat is uncommon *In the next 24 hours the fever reaches* a sustained level The nose may be obstructed The throat feels dry and full Laryngitis with hoarseness and a hacking, unproductive cough is frequent, substernal soreness may be noted These manifestations probably reflect the effect of the virus on the lower respiratory mucosa There is little appetite, but nausea, vomiting and diarrhea are not common features At the height of the uncomplicated disease physical findings are few The patient is apathetic *The face is flushed, and the* conjunctivae are suffused The nasal mucous membranes are frequently bright red, and areas of *hemorrhage may be noted* *The* tongue is moderately coated The nasopharynx, the oropharynx and the soft palate are red and glazed with a shiny mucoid coating The lymphoid follicles, particularly those on the soft palate, are usually enlarged and dewy in appearance Purulent exudate is not seen on the tonsils or the pharyngeal wall Muscular soreness, *occasionally* hyperesthesia, may be detected In perhaps a third of the patients scattered sibilant mucous rales *may be heard over the larger bronchi*, but evidence of pulmonary involvement is otherwise absent The heart rate is variable, slight tachycardia is common; or a relative *bradycardia may be noted* *Blood pressure may be* somewhat lower than normal The abdomen is usually negative Splenomegaly is not ordinarily encountered Signs of encephalitic disturbance are reported but uncommon except as they reflect febrile reactions The leukocyte count ranges from 6,000 to 8,000, but in about one third of cases leukopenia occurs *A relative increase of lymphocytes is noted* Leukocytosis can be considered as evidence of complications Urinary findings are ordinarily limited to some febrile albuminuria Bacteriologic blood cultures are negative Bacteriologic examination of the throat has revealed no consistent bacterial accompaniment of influenza virus infection, however, the presence of *significant pathogens* in the cultures should be considered an additional risk to the patient In the ordinary case roentgenograms of the chest show little, some increases in hilar shadows may be noted

There may be some fluctuation in temperature, but 3 to 4 days after onset it falls sharply or declines to a normal range A slight afternoon rise may be noted for another day or two Even in severe outbreaks the great bulk of cases behave as mild "3-day fevers" Convalescence ordinarily proceeds rapidly, but annoying cough often persists for some time Despite the apparent recovery, distressing features of the disease may appear when the patient undertakes full activity too soon Fatigue and weariness are often troublesome, this is less pronounced in children and young adults with their greater reserves *In older persons it may be a prolonged course* Mental depression and difficulty in concentration may be marked The patient may experience palpitation, especially on exertion, rapid pulse or bradycardia may continue The nature of this disturbance is not known but it, too, suggests a form of "toxic" injury like that of the constitutional reaction

Pulmonary complications are the most common They are to be suspected if temperature and *symptoms persist beyond* the 4th or the 5th day There may be tracheitis, with increased substernal tightness or pain and painful cough with thick, tenacious sputum Dyspnea and cyanosis may develop Bronchitis,

bronchiolitis or pneumonia may supervene. Since these conditions are commonly associated with bacterial infection, the pulmonary signs are influenced by the nature of the bacterium as are the x-ray findings. In other instances, the temperature and the symptoms may have subsided, and the patient has begun to move about when a chill and sudden rise of temperature occur with a rapid return of symptoms Frequently, these episodes have been referred to improperly as relapses, as if of the primary illness, but they, too, usually represent the onset of bacterial pulmonary complications Leukocytosis develops, blood cultures may be positive, and examination of the sputum provides information of the complicating organism. The course will depend on the severity and the nature of the bacterial agent and the response to therapy Diffuse bronchopneumonia, lobar pneumonia, empyema or pneumothorax may occur. In a small proportion of cases a fulminant pneumonia ensues. The patient may appear to have a characteristically mild illness for 1 or 2 days when, acutely, extreme dyspnea and prostration develop Within a matter of hours the patient is dangerously ill and presents a shocked appearance with livid or heliotrope cyanosis, sweating, tachycardia, feeble pulse and low blood pressure Temperature may fall below normal Cough is feeble, and a thin serosanguinous fluid may exude from the respiratory tract Instances have been noted in which fluid really ran from the lung when the head was placed in a dependent position The physical signs in the chest may be very misleading Diffuse rales, more prominent at the bases, are heard, breath sounds are irregularly suppressed or perhaps exaggerated. The picture is really that of inflammatory pulmonary edema Roentgenograms may reveal patchy opacities or a diffuse haziness in the lower lung fields The most common organism associated with the virus infection in these cases has been a coagulase positive staphylococcus pyogenes Although acute staphylococcal infection of itself may produce a severe pneumonitis with diffuse involvement, in the presence of viral injury an additive effect occurs. Rapid deaths of somewhat similar clinical course also occur when other organisms are encountered. In some instances no bacterial component has been identified, and in epidemics associated with virulent strains of virus it seems likely that virus alone may cause these severe illnesses as in experimental animals. Persons with mitral stenosis appear to be especially vulnerable as if the development of alveolar fluid increased the probability of decompensation and further exaggeration of the pulmonary difficulty. Patients with other forms of advanced heart disease or chronic pulmonary fibrosis may tolerate the disease poorly.

Significant pneumonic complications among cases from the general population in usual epidemics scarcely exceed 1 per cent of the total. They are more frequent in young children and people over 50 years of age. In the epidemic of 1889-90 the incidence of pneumonia among cases of influenza was estimated to be 5 per cent. But in the severe pandemic of 1918 it was still higher, and in certain military installations an incidence of 20 to 30 per cent of pneumonia was noted with a case fatality in some as high as 30 per cent (Jordan 1927, Hall, 1928) Moreover, in 1918 an excessively high frequency was noted in persons of 20 to 40 years of age, particularly among males Obviously, the increased incidence of influenza results in an increased frequency of pneumonia at all times.

Influenza acquired during pregnancy often results in miscarriage or abortion It seems probable that congenital defects are also increased. There is an increased maternal and infant mortality so that influenza is a serious complication of pregnancy.

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PATHOLOGIC PICTURE

Most of the information as to the pathologic changes occurring in influenza of man is derived from fatal pneumonia cases in which bacterial invaders play a prominent role As a result, a great complexity of pictures is described from which the viral injury must be extrapolated However, studies in experimental animals with influenza viruses have resulted in a clear picture of the primary viral injury Influenza virus exerts a selective injury on the ciliated respiratory epithelium Within 48 hours after infection of a ferret with a well-adapted strain, the nasal respiratory epithelium is largely destroyed, leaving only the germinative basement membrane The adjoining olfactory and squamous epithelia are essentially unaffected (Francis and Stuart-Harris, 1938) Regeneration begins rapidly from the basement layer, and by the 6th day a transitional type of epithelium composed of polyhedral cells 3 to 4 layers deep is rather uniformly present Repair proceeds in the next week through stages of stratified squamous, hyperplastic columnar and the return of relatively normal ciliated columnar cells excreting mucus However, islands of cells which have not completely differentiated may still remain Studies of the lower respiratory tract have shown in ferrets (Francis, 1934, Shope, 1934, Brightman, 1936), swine (Shope, 1936), and mice (Straub, 1937) that the earliest lesion is the necrotizing damage to the respiratory ciliated epithelium The earliest injury may be more consistent in the bronchioles than in the trachea and the primary bronchi (Stuart-Harris, 1953). The epithelium of the terminal respiratory bronchioles is made up of cuboidal nonciliated cells which do not secrete mucus, this tissue is not generally destroyed The severity of epithelial injury is undoubtedly influenced by the virulence of the strain

In ferrets and mice extensive pulmonary involvement develops about the hilum and extends peripherally, involving primarily the dependent lobes Entire lobes or even most of the lung may be affected The pleura is smooth, glistening and taut Grossly, the affected portions of the lung are distended and firm, purplish in color and filled with edema fluid Large amounts of serous fluid exude from the cut surface and from the bronchi after which the tissue becomes wrinkled and rubbery

Microscopically, in addition to irregular epithelial necrosis, the alveolar walls are swollen, and the spaces are distended with edema fluid and hemorrhagic extravasations The cellular exudate is a scanty distribution of large pale-staining mononuclear cells There are hyperemia and edema of the bronchial and bronchiolar tunica Peribronchiolar collections of round cells are seen Necrotic exudate is present in the lumen There is dilatation of the blood vessels, edema of their walls and some perivascular cuffing The character of the pulmonary lesion is that of a profuse edema secondary to the epithelial injury rather than an acute inflammatory pyogenic pneumonitis With recovery, epithelial hyperplasia and regeneration proceed more slowly than in the turbinates Nodular hyperplastic overgrowths are prominent These pneumonias are bacteria-free and are clearly related to virus alone Shope's studies have shown the marked differences in severity between a mild filtrate disease produced by swine influenza virus alone and the extensive pulmonary involvement when virus and *H influenzae suis* are combined (Shope, 1936). Moreover, in ferrets and mice it has been noted repeatedly that much more severe disease may occur in the presence of chronic bacterial infection of the animal, the pathologic pictures contain the basic viral lesions but they may be largely obscured by the extensive changes resulting from bacterial invasion.

Although the pathologic picture of influenza virus infection in man is clouded by bacterial complications, certain investigators of pandemic fatalities called attention to epi-

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alveolar walls which are not primarily affected by influenza virus Repair is much delayed, and fibrous replacement occurs Unfortunately, no cases of streptococcus pneumonia were investigated Sections from fatal cases of 1918 were also studied. Staining revealed staphylococci in 7 of the 8 instances where bacteria could be identified The character and the extent of epithelial degeneration

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Hers also demonstrated that the epithelial lesions associated with influenza virus are also found in measles and varicella This similarity with measles was emphasized by MacCallum (1919) in studies of pneumonia associated with measles in 1917 See also Callender (1929)

Because of the concentrated attention given to the autumnal epidemic of 1957, numerous fatal cases were reported to the U S P H S A number of them were staphylococcal; from others no significant bacteria were recovered, although this feature is complicated by antibiotic therapy A significant proportion had mitral disease or were pregnant Extensive edematous pneumonia is described, sometimes with hyaline membranes (CDC Influenza Reports, 1957-58)

into the pancreas and the ovary have been considered not infrequent Hemorrhage into the adrenals occurs frequently in the presence of staphylococcal complications and may play a role in the picture of physiologic collapse Ordinarily, the central nervous system has displayed no evidence of primary injury Although encephalitis lethargica was prevalent in 1918, no causal relation to influenza virus has been shown

EXPERIMENTAL INFECTION, HOST RANGE

Although there had been earlier reports of the transmission of a virus from influenza of man to experimental animals, knowledge of its etiology begins with the demonstration that a virus could be transmitted intranasally to ferrets with filtrates of garglings from influenza patients Then the infection could be maintained in serial passage with extracts of ferret's turbinate tissue (Smith et al, 1933) Characteristically, after an incubation period of 48 hours there is an abrupt onset of fever with lassitude, sneezing, yawning, nasal discharge and loss of appetite. The eyes are congested, and the fur is ruffled or matted Virus is essentially localized in the nasal tissues Generally, the evidence of illness lasts no more than 4 to 5 days, the animal recovers rapidly and is immune to reinfection at that time Virus disappears, and specific antibodies rapidly appear in the blood If ferrets are etherized at the time of inoculation, the disease becomes progressively more severe in passage as the virus becomes adapted to the lungs with the production of extensive, often fatal, viral pneumonia (Francis, 1934). Virus is readily recovered from the lungs as well as from the nose. It has been noted that virus inoculation of ferrets which are maintaining a naturally acquired respiratory infection with β hemolytic streptococci or pasteurella may exhibit a form of disease still further enhanced by invasion of the existing bacterial pathogens Recovery from virus infection does not result in permanent immunity, and in a few months intranasal inoculation of the same virus may induce febrile illness with damage again to the nasal respiratory epithelium. Usually the lungs are not involved again

There is considerable difference in the capacity of different strains of Type A to elicit fever and clinical evidence of infection and in their adaptability to produce significant pulmonary disease Type B strains produce mild disease, generally, and become adapted slowly Multiplication of Type C strains appears to be limited to the turbinates, nevertheless, the development of antibodies reflects the infectious experience Limited experience with Type D (Sendai) virus resulted in fever, lung consolidation, and death with early ferret passages, but milder illness occurred in later passages (Jensen et al, 1955)

The various forms of influenza in the ferret closely resemble those of man They are highly susceptible and also produce high yields of virus Ferrets may become infected by natural exposure outside the laboratory, and this may

thelial degenerations at all levels of the respiratory tract apart from purulent necrotizing bacterial injury Moreover, they were noted in pneumonias associated with bacteria which do not ordinarily cause primary degeneration of ciliated epithelium Rapid regenerative hyperplasia and metaplasia were also commonly observed Thus, the epithelial lesion was suggested as characteristic of influenzal infection. In addition, 2 notable cases of 1918 were reported by Goodpasture and Burnett (1919) in which epithelial lesions were noted in the absence of demonstrable bacteria There can be little doubt, however, that a major part of the pneumonic pathology of 1918 was related to the nature of the bacterial component Its character varied in time and location of the study. Opie et al (1921) repeatedly emphasized the change in nature of the pulmonary disease in the course of the epidemic with the advent of a new bacterial pathogen and noted that the individual patient might suffer a succession of pneumonias. Hence, the features of lobar or lobular pneumococcal pneumonia and empyema, of *H. influenzae* interstitial bronchiolitis, of β hemolytic streptococcal interstitial suppurative bronchopneumonia and early serous empyema, of staphylococcal necrotizing bronchitis with miliary abscesses were commonly reported as preponderant in various areas The pathologic picture characteristic of a given bacterium became more prominent with the duration of the disease. But in a certain proportion of cases succumbing early in the disease there was what Winternitz et al (1920) called "acute, diffuse, influenzal pneumonia" The descriptions are striking The dependent portions of the lung are most frequently involved There

There may be little true consolidation Marked destruction of the tracheal and the bronchial epithelium is described. The bronchioles are distended and contain exudate, the alveolar walls are thickened Hemorrhage into the alveoli is prominent, but exudate is scanty and aplastic One feature was considered by some to be characteristic: the alveolar ducts are dilated and completely lined by a hyaline membrane in the center of which an air bubble can be seen Goodpasture (1919) showed that it did not take the fibrin stain It resembles a concentrated amorphous exudate pushed by respiration from the bronchioles against the surface of the alveolar walls The nature of *this material is* not known, but it has not been noted in experimental disease. This extremely acute picture was ascribed by different investigators to different bacterial agents, and that fact, of itself, indicates that the outstanding feature of extensive edema was probably a consequence of the basic viral injury as it is in experimental disease. Thereby the different organisms were provided a rich medium in which

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fluenzal virus-pneumococcal pulmonary infection of rats

Since the identification of influenza viruses, studies of fatalities occurring during epidemic periods have been limited However, they have emphasized the fact that the majority of fatal cases with demonstrated influenza virus infection have been associated with coagulase positive *Staphylococcus aureus* (Scadding, 1937; Parker et al, 1946; Straub and Mulder, 1948; Mulder and Verdonk, 1949, Stuart-Harris et al., 1950; Hers and Mulder, 1951, 1953) Because of destructive effect of staphylococcal infection, query has been raised as to the relative importance of virus and coccus in production of the epithelial injury. Hers and Mulder (1951) and Stuart-Harris (1953) have reported recovery of virus from the lungs of fatal cases in which no epithelial lesions could be demonstrated On the other hand, Hubble and Osborn (1941) described the typical lesion in 2 cases dying during an epidemic in 1940, from whose lungs no bacteria were recovered This entire problem has been studied beautifully by Hers (1955) who has compared by detailed histologic and microbiologic technics the pathology in fatal cases of bacterial pneumonia of 4 to 12 days' duration, with or without associated influenza A viral infections He has demonstrated that epithelial degeneration does not occur with *H. influenzae* or pneumococcus unless influenza virus infection is also present The basal cell layer of the epithelium remains intact Interstitial extension in the bronchioles and the lung is largely absent, as also are hyperemia and edema and vascular thrombi. The same viral lesion occurs in conjunction with staphylococcus, but there the wounded epithelium becomes invaded, the basal layer is seriously damaged, and colonies of bacteria form abscesses in the tunica so as to push out plugs of exudate into the lumen Hyperemia, edema, hemorrhage and vascular thrombi are extreme Moreover, the destructive lesions extend into the respiratory bronchioles and

alveolar walls which are not primarily affected by influenza virus Repair is much delayed, and fibrous replacement occurs Unfortunately, no cases of streptococcus pneumonia were investigated Sections from fatal cases of 1918 were also studied Staining revealed staphylococci in 7 of the 8 instances where bacteria could be identified The character and the extent of epithelial degeneration and regeneration did not differ significantly between them and cases of 1949-53, nor was the hemorrhagic edema of different degree Hers also demonstrated that the epithelial lesions associated with influenza virus are also found in measles and varicella This similarity with measles was emphasized by MacCallum (1919) in studies of pneumonia associated with measles in 1917 See also Callender (1929)

Because of the concentrated attention given to the autumnal epidemic of 1957, numerous fatal cases were reported to the U S P H S A number of them were staphylococcal, from others no significant bacteria were recovered, although this feature is complicated by antibiotic therapy A significant proportion had mitral disease or were pregnant Extensive edematous pneumonia is described, sometimes with hyaline membranes (CDC Influenza Reports, 1957-58)

Significant pathologic changes in other organs have not been consistently observed, and when present their relation to associated bacterial infection is not readily assessed The vascular leakage observed with influenza virus infection does suggest that either directly or indirectly the virus has an effect on vascular permeability Certain hyaline muscular changes have been described Hemorrhages into the pancreas and the ovary have been considered not infrequent Hemorrhage into the adrenals occurs frequently in the presence of staphylococcal complications and may play a role in the picture of physiologic collapse Ordinarily, the central nervous system has displayed no evidence of primary injury Although encephalitis lethargica was prevalent in 1918, no causal relation to influenza virus has been shown

EXPERIMENTAL INFECTION, HOST RANGE

Although there had been earlier reports of the transmission of a virus from influenza of man to experimental animals, knowledge of its etiology begins with the demonstration that a virus could be transmitted intranasally to ferrets with filtrates of gargings from influenza patients. Then the infection could be maintained in serial passage with extracts of ferret's turbinate tissue (Smith et al, 1933). Characteristically, after an incubation period of 48 hours there is an abrupt onset of fever with lassitude, sneezing, yawning, nasal discharge and loss of appetite The eyes are congested, and the fur is ruffled or matted Virus is essentially localized in the nasal tissues Generally, the evidence of illness lasts no more than 4 to 5 days, the animal recovers rapidly and is immune to reinfection at that time Virus disappears, and specific antibodies rapidly appear in the blood If ferrets are etherized at the time of inoculation, the disease becomes progressively more severe in passage as the virus becomes adapted to the lungs with the production of extensive, often fatal, viral pneumonia (Francis, 1934). Virus is readily recovered from the lungs as well as from the nose. It has been noted that virus inoculation of ferrets which are maintaining a naturally acquired respiratory infection with β hemolytic streptococci or pasteurella may exhibit a form of disease still further enhanced by invasion of the existing bacterial pathogens Recovery from virus infection does not result in permanent immunity, and in a few months intranasal inoculation of the same virus may induce febrile illness with damage again to the nasal respiratory epithelium Usually the lungs are not involved again

There is considerable difference in the capacity of different strains of Type A to elicit fever and clinical evidence of infection and in their adaptability to produce significant pulmonary disease Type B [illegible]

development of antibodies reflects the infectious experience Limited experience with Type D (Sendai) virus resulted in fever, lung consolidation, and death with early ferret passages, but milder illness occurred in later passages (Jensen et al, 1955)

The various forms of influenza in the ferret closely resemble those of man They are highly susceptible and also produce high yields of virus Ferrets may become infected by natural exposure outside the laboratory, and this may

cause great difficulty in virus passage Their susceptibility to laboratory exposure to influenza virus and to distemper virus requires their strict isolation

Adaptation of influenza virus to mice was valuable for investigation (Andrewes et al, 1934, Francis, 1934) and diagnostic procedures Intranasal instillation of virus from ferret or man into lightly etherized mice and repeated passage at 2- to 3-day intervals with suspensions of mouse lungs commonly results in progressive adaptation of the virus to produce fatal pulmonary disease The time of death is a fair measure of the virus concentration administered With large doses of virus, deaths may occur in 2 to 3 days, with smaller amounts, in 10 to 14 days The lung lesions or fatality provide useful end points in demonstration of virus-neutralizing antibodies and for studies of pathogenesis and immunity The involved lungs are swollen, edematous and plum colored; the entire lung except for small emphysematous margins may be involved, grading to small lesions in nonfatal infection Highly adapted strains given intraperitoneally may cause pulmonary lesions, apparently by overflow from a transient viremia into the upper air passages (Rickard and Francis, 1938) After intravenous inoculation, virus may be recovered from the lung and appears to multiply without producing significant lesions, this apparently is related to the presence of virus on the vascular rather than the epithelial side of the vulnerable tissues. Although influenza virus can be isolated directly in mouse lung, its early recognition requires transfer to eggs Adaptation to produce suitable pulmonary lesions may be a slow process Influenza B strains are usually less virulent and have lower titers in mice than A strains. Studies of the growth curve of a well-adapted strain of Type A in mice have indicated a generation cycle of a few hours and the peak of virus production in 48 hours, thus preceding the development of pulmonary lesions (Taylor, 1941) Type C strains have not been adapted to the lungs of mice; as in ferrets, the virus remains limited to the turbinates Type D virus (Sendai) is a source of uncertainty. Japanese authors report its recovery from mice. With passage it produces fatal pulmonary disease, but death does not occur until about the 7th day, and virus titers are low. The WS strain of Type A virus became rapidly adapted to intracerebral virulence in mice and can be maintained as a neurotropic strain (Stuart-Harris, 1939, Francis and Moore, 1940).

Mice can be readily immunized by the intraperitoneal route with active or inactive virus Antigenic content of a vaccine may be quantitated by this procedure Subcutaneous vaccination is less effective. Recovery from infection gives immunity to a wide range of strains of the same type, but there is no specific cross immunity with other types

The chick embryo is a most susceptible host New strains are best isolated in the amniotic sac of 13- to 14-day embryos (Burnet, 1940; Beveridge and Burnet, 1946). Direct isolation can be made also by inoculation into the allantoic cavity, preferably of 9- to 10-day embryos, but this is a less sensitive procedure (Hirst, 1945). After a few amniotic passages most strains will propagate satisfactorily in the allantoic cavity The virus multiplies in the embryonic membranes and is given off into the extra embryonic fluids reaching infectious titers of 10^{-9} (Pearson, 1944) The virus also exhibits its characteristic tropism in the destruction of the respiratory epithelium of the

are incubated for 2 to 4 days, and the presence of virus is recognized by agglutination of erythrocytes. Ordinarily, about 10^6 infectious units are required to cause visible agglutination of erythrocytes The virus may be identified by the inhibiting action of specific immune serum Type C strains grow well in the amniotic sac but irregularly in the allantoic cavity

Strains that have been passed repeatedly often develop the capacity to cause death of the embryo in 2 to 3 days, the time of death is related to the concentration of virus However, the Asian strains of 1957 induced this from the start in a goodly proportion of embryos

Infection can be established on the chorioallantoic membrane or by injections into the yolk sac. These procedures are less satisfactory and are employed essentially for special purposes A de-embryonated preparation, i e, the membranes remaining in place after re-

moval of embryo and fluids, has proved to be of great value in studies requiring repeated measures of virus production (Bernkopf, 1950).

Influenza virus was first cultured in vitro, using the medium of Li and Rivers, i.e., minced chick embryo tissue in Tyrode's solution (Francis and Magill, 1935). Virus can be maintained in serial passage indefinitely and retains a significant pathogenicity for ferrets and mice. Tissue culture can also be used for initial isolation of virus from man. Minced embryo tissue on nutrient agar or in roller tubes can also be used (Pearson and Enders, 1941). In recent years whole chorio-allantoic membranes in balanced salt solution in a respirometer flask have been extremely useful in studying viral synthesis and its inhibition (Ackermann, 1951). In cultures of chick-embryo lungs or monkey kidney cells some strains have been observed to produce cytopathic injury with plaque formation (Ledinko 1955. Henry and Youngner, 1957). Use of the chorio-allantoic membrane in culture has provided additional evidence of the mucoid nature of virus receptors in that tissue, of their destruction by virus and their regeneration (Stone, 1948). Moreover, the release of virus has been demonstrated to proceed in a relatively continuous manner for hours rather than as an explosive burst, several generations may be released at intervals of 5 to 6 hours from the same tissue—although it is not certain they are from the same cells (Ackermann and Maassab 1954a). Nevertheless, the release from single cells is observed to occur over a period of time (Cairns, 1952). When large doses of virus are employed to introduce virus into all cells, the first release of virus occurs between 2 and 3 hours, it continues at a rapid rate for the next 7 hours, and by 12 hours is essentially complete, although lesser amounts of virus may be forthcoming for several hours later (Ackermann and Maassab, 1954a). The cells obviously are not rapidly destroyed. This relatively simple tissue behaves differently from the highly differentiated respiratory epithelium in that respect. The lag period of Type D virus is 6 to 7 hours in similar cultures, and the release is slower (Jensen et al., 1955, Ackermann and Maassab, 1954b).

Influenza viruses have been shown to damage respiratory epithelium maintained in cultures (Bang, 1958). Type D virus has been observed to produce cytopathic effect on cultures of human embryonic lung in roller tubes, while strains of other types did not (Gorbunova et al., 1957).

Infection can be induced experimentally in man by intranasal instillation of virus that has been maintained in ferrets, mice, or eggs. The nature of the illness differs little from that naturally acquired. Definite signs of pneumonitis may develop. It has been suggested that because of the large inoculum used some of the illness is a "toxic" reaction rather than infection. This is a dubious conclusion, for subjects given equal concentrations of inactivated virus do not exhibit signs or symptoms of illness, nor do those persons who resist the challenge with active virus. On the other hand, the PR8 strain passed in tissue culture was noted to be so attenuated as to excite no illness in adults, and antibody response was infrequent (Francis, 1940b, 1950). Both influenza A and influenza B have been experimentally induced in man by various investigators, this has been reviewed earlier (Francis 1950). An effort to incite influenza C in adults was not successful (Quilligan et al. 1954). Japanese investigators have reported the induction of disease in human subjects with Type D virus (Kuroya et al., 1953, Ishida 1957). Introduction of swine influenza virus, after repeated tissue culture, intranasally into a group of men caused little illness. Russian scientists have had extensive experience in experimental infection of volunteers in relation to the development and the use of active virus vaccine (Smorodintsev and Zhdanov 1957).

Swine have been a continued host for swine influenza virus. Shope has demonstrated that the characteristic disease seen in swine herds is a joint infection by virus and *H. influenzae suis*, although spread of the disease and immunity thereto is essentially related to the virus alone (Shope 1934, 1936). Shope has also described experiments indicating that the lung worm of hogs may be an intermediate host in which virus in a "masked" form is maintained between epidemics. The lung worm apparently becomes host to the virus when in the hog's lung. the larvae excreted onto the soil are ingested by earthworms and undergo additional stages of development

The earthworms are eaten by hogs, the lung worm larvae migrate from the alimentary tract to the lung, all this time retaining virus in a masked form demonstrable only when certain provoking stimuli—cold, various disturbing injections—break out the virus and incite influenza in the swine host (Shope, 1941a, b; 1943a, b, 1954). In other countries strains of virus more closely similar to other strains isolated from man have been recovered from swine influenza, and *H. influenzae* has been found less regularly Swine influenza virus was noted by Shope to produce pulmonary disease in mice and ferrets from the initial passage. Evidence has been gained of infection of swine in nature with Type A viruses prevalent in the human population at the time (Shope, 1938). Recovery from swine of the human epidemic strain of 1957 was reported from Japan (WHO bulletin). Type D virus has also been reported in outbreaks of disease in swine (Sasahara, 1955)

Types A and B viruses have been adapted to hamsters A number of other species, including certain squirrels, mink and chipmunks, have been shown to have a degree of susceptibility. Monkeys may show evidence of inapparent infection

Horse influenza has not been associated with viruses resembling the influenza group However, Sovinova et al (1957) have described an outbreak of respiratory disease in horses from which a virus related by complement-fixation test to Type A influenza virus was recovered Serologic studies revealed the development of specific HI as well as CF antibodies with recovery In HI tests no significant crossing with other strains of Type A has been noted The virus causes mild illness in ferrets after egg passage Antibodies in the human population of the U S A have not been demonstrated, but certain individuals recovering from Asian 1957 strains show low serologic crossing with this equine strain by complement-fixation tests (Maassab and Francis, 1958).

One factor which must be seriously considered when influenza viruses are said to be isolated from odd species of animals is possible laboratory contamination This has caused confusion in the past. If a presumedly new strain produces pulmonary disease in ferrets and mice from the start, it should be suspect. On the other hand, one may observe limited lesions in the lungs of animals when throat washings are inoculated, but they do not persist in the next transfers

ETIOLOGY

The primary causative agents are viruses of medium size At present the influenza viruses of human origin are divided into 3, probably 4, immunologically distinct serologic types, i e, A, B, C, D. Types A and B have been shown to comprise numerous variant strains which tend to group themselves serologically into families with closer resemblances among the familial members than to strains of the other groups. The virus of swine influenza described by Shope (1931) belongs to Type A; certain other strains recovered from infection in hogs have also been of Type A A classification on this basis might be as follows.

INFLUENZA VIRUS, TYPE A

Group	Prototype	Common Term	Prevalence
A_1	S15	Swine influenza	19— to 1928
A_2	PR8	Influenza A virus	1934-43
A_3	FM1	A prime virus	1946-57
A_4	Japan 305	Asian influenza	1957 (188?-189?)

The original WS strain of 1933 is difficult to classify; age distribution of antibodies in human population indicates that it is chronologically similar to PR8 Similar variations of Type B have been observed but less sharply distinguished. Nevertheless, 2 subgroups may be suggested·

INFLUENZA VIRUS, TYPE B

Group	Prototype	Common Term	Prevalence
B_1	Lee	Influenza B virus	1936-48
B_2	GL	—	1954-

These divisions are less certain, there are more intermediate strains as shown by age

distribution of antibodies, and the points of division may differ in various regions. The present division is based on the fact that vaccination with Lee strain was effective in epidemics of 1945 and 1952 but of limited influence in 1954-55.

There is no information of significant variants among Type C or D strains The type character is readily demonstrable by complement-fixation, and the strain or group variation within a type can be demonstrated best by other serologic procedures Cross-reactions between the types do not occur, but Schafer (1955) has reported crossing in complement-fixation with nucleic acid fractions of fowl plague virus and FMI strain of Type A influenza virus

Influenza viruses have been described as round or ovoid elementary bodies, discrete particles of relatively uniform size, measuring 80 to 120 mμ in diameter, Type B virus is somewhat larger than Type A (Elford et al, 1936, Sharp, Taylor et al, 1944, Friedewald and Pickels, 1944) Preliminary data suggest that Type D virus is of irregular size and may be 200 mμ in diameter (Hartman and Ackermann, 1954, Nishikawa and Fukumi, 1954) There are pleomorphic filamentous forms of influenza virus of 1 mμ or more, most commonly seen with newly isolated strains (Mosley and Wyckoff, 1946, Dawson and Elford, 1949). They have been detected in tissues infected with old passage strains as well (Wyckoff, 1951, Murphy and Bang, 1952) They can be observed in darkfield preparations, extensive studies of their formation by electronmicroscopy suggest that they are viral structures assuming different morphology as a result of some defect in their emergence through the cell wall Their infectious capacity is still uncertain, but they retain specific antigenic components They can be broken down into smaller units by physical and chemical procedures, and numerous suggestions have been made that the spherical bodies are so derived Morgan et al (1956) disagree sharply with that interpretation and consider the 2 forms as being independent, the spherical bodies they believe to be infectious and the filaments noninfectious Infectivity of virus preparations is lost following heating at 56° C for a few minutes, after ultraviolet irradiation, sonic vibration, treatment with formaldehyde, long-chain fatty acids and numerous other reagents Maximum stability of biologic properties is maintained between pH 6 5 and 7 9, their loss is more rapid in the acid range than in the alkaline The iso-electric point for the PR8 strain is pH 5.3 (Miller et al, 1944). As with many other agents, they are vulnerable to physiologic saline, but the addition of various proteins makes a relatively stable medium Purified preparations, properly buffered, may be held at 4° C for a month without showing marked reduction in titer Virus in allantoic fluid may be similarly maintained Ordinary freezing is harmful, but suspensions stored at −70° C for many months maintain their titer, especially if stored in sealed glass ampules The viruses are also well preserved when dried in vacuo

Influenza viruses display a variety of activities Foremost of these is infectivity which exhibits a selective affinity for epithelial cells of the respiratory tract in all affected species The effect is most consistent in damage to the nasal respiratory cells, but with well-adapted, virulent strains it extends to the ciliated respiratory epithelium of the trachea and the bronchial tree The WS strain has been shown to be adaptable as well to the brain in mice (Stuart-Harris, 1939, Francis and Moore, 1940) Certain strains exhibit the capacity to produce fatal hemorrhagic disorder of chick embryos, indicating an effect upon the vascular endothelium Ivanovics et al (1954) have reported the adsorption and the elution of virus from the vascular endothelium of guinea pigs, rats and mice However, influenza virus is essentially a pneumotropic agent

Influenza viruses were the first among animal viruses to be shown to possess the capacity to cause agglutination of red blood cells (Hirst, 1941, McClelland and Hare, 1941) Others, including mumps, Newcastle disease and fowl plague viruses, behave similarly Hemagglutination is the result of the interaction of the virus and the surface of erythrocytes, the second reaction is the agglutination of red-cell-virus complexes, the third is the spontaneous disassociation of the virus from the red cells Apparently a single virus particle may cause clumping between 2 erythrocytes (Horsfall, 1954) Hirst (1942) demonstrated that the virus released from the cells retains its hemagglutinating capacity

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is not infective; it does not cause hemagglutination, nor does it elicit immunity. The antigen is common to all strains of a given type but does not cross with those of other types. Antibody to the soluble antigen develops generally only after infection. The use of complement-fixation with soluble antigen has certain distinct advantages in diagnostic work, especially for identification of the type of virus when strains of aberrant antigenic character are encountered. It possesses possible theoretical advantages in the diagnosis of illness in vaccinated persons when interpretation of hemagglutination-inhibition results may be complicated by antibodies derived from vaccination.

Strains of Types A and B cause a toxic injury following intracerebral, intraperitoneal or intravenous injection of virus in mice, guinea pigs, rabbits, rats and hamsters (Henle and Henle, 1946a, b). The toxic property cannot be separated from the infective property; toxic activity is not the result of virus multiplication. Toxic activity is directly correlated with virus concentration. Intraperitoneal or intravenous injection in mice may cause death in from 8 to 96 hours. The toxic effect of influenza A virus preparations is neutralized by anti-influenza A serum but not by anti-B virus serum, and vice versa. Moreover, vaccination of mice by either the subcutaneous or the intraperitoneal route causes them to become specifically immune against the toxic effects of the same serologic type. The toxic property remains stable on storage at 4° C. for 2 or 3 months and is inactivated by heating, treatment with formaldehyde or irradiation with ultraviolet light at a rate slower than the infectivity. It is thought that the virus particle itself carries the toxic property (Henle and Henle, 1946b).

Another form of toxic reaction is observed after subcutaneous injection in man, its severity is directly related to the amount of virus (Salk, 1948, Quilligan et al., 1948). The reaction occurs with inactivated virus. It is most severe in young children who may have high fever, chills and mild delirium. There is usually well-marked inflammatory reaction at the site of injection. In experimental animals it behaves as a pyrogen, small amounts may desensitize the animal so that later it will tolerate otherwise fatal doses.

Consideration of the formation of influenza virus has sought to explain the origins and the nature of the hemagglutinin and the soluble antigen. Study of the development and the growth of virus has revealed a period after virus enters the cell when it apparently loses its identity followed, after an interval of 2 to 3 hours, by the appearance in tissues of soluble antigen and hemagglutinin which is not infectious, while mature infectious virus appears later. When large amounts of virus are used to inoculate the membranes in deembryonate eggs or tissue cultures (Bernkopf, 1950, Henle and Girardi, 1955), the virus produced has relatively high hemagglutinating titer but a low infectious titer. It appears that virus has been produced in a form whose functional development is incomplete. Von Magnus (1947) originally observed this phenomenon, repeated passage with high-titer inoculum resulted in a progressive reduction of infectious virus while hemagglutinin titers remained high. Extensive study of the phenomenon has not provided full explanation, but it seems certain that overloading of the available cells and the presence of noninfectious virus in the inoculum play a role in incomplete formation of virus material. In fact, it seems likely that most virus yields contain particles of that nature. They are not infectious and probably not effective immunizing antigens. However, they do combine with cells and of themselves may restrict the yield of complete virus. Soluble antigen is also produced in large amounts. This area of investigation has been reviewed extensively (Henle, 1953, Von Magnus, 1954, Finter et al., 1955). Hoyle, in his analysis, has concluded that the virus particle after treatment with ether is disrupted with the liberation of 2 smaller particles. (1) the "soluble" antigen, contains essentially all the ribonucleoprotein (5.3 per cent RNA), or about 14 per cent of the weight of the infective particle, (2) the hemagglutinin is considered a mucoprotein, totaling 13 per cent of the particle, with the protein carrying the combining group having affinity for cells and also possessing the enzymic activity. It was estimated that 38 per cent was nonprotein material, 3.5 per cent of it carbohydrate, and 34.5 per cent lipid; in addition, 34 per cent other protein is com-

while the cells can no longer combine with the same or closely related strains. The fact that different viruses may combine with cells previously acted upon by a given virus gave rise to the effort to classify them in a "receptor gradient" series (Burnet et al, 1946) The cells are altered so that they migrate differently in an electrophoretic field (Hanig, 1948) Hirst emphasized the enzymatic nature of the reaction and that the substrate was a mucoprotein on the surface of the erythrocytes He showed that virus is adsorbed and subsequently released spontaneously from the cells of the excised ferret lung (Hirst, 1943). Attachment and enzymatic effects have been observed with mouse lung tissues, chorio-allantoic membrane, vascular endothelium of different species; virus combines with leukocytes *in vitro* and reduces their phagocytic capacity (Merchant and Morgan, 1950) Recent study has shown that virus particles are themselves phagocytized by leukocytes (Boand et al, 1957; Hanson et al, 1957) The suggestion from all these observations is that the enzymatic activity is an essential component of the process by which influenza virus gains entry to the susceptible mucus-producing respiratory epithelium to establish infection.

Interference is demonstrable with influenza as with many other viruses It is apparently related to the saturation of receptor sites or essential metabolic processes by one strain *so that another cannot be satisfactorily established* in the same cells Interference has been demonstrated in tissue cultures, eggs, mice and ferrets Cross-effect between Type A and Type B strains has been demonstrated, but this may be overcome by increasing the amount of the second virus. Large amounts of virus rendered noninfectious by ultraviolet light may establish interference. The effect is apparently induced by the virus particle itself Interference between influenza and a number of other viruses has been demonstrated The subject has been reviewed by Henle (1953) Recent studies purport to indicate that a product of cell-virus interaction called "Interferon" plays a role in interference (Lindemann et al, 1957).

Studies of virus-cell interaction have revealed the occurrence of numerous mucoproteins in body fluids or tissues and in ovo*mucin which react with the virus and inhibit* its combination with erythrocytes in vitro They appear to represent the normal substrate upon which these viruses act The inhibitors are destroyed by continued exposure *to the active* virus, leaving the virus again able to act in a normal manner (Hirst, 1942a). If virus is heated at 56° C. to de*stroy infectivity, the capacity to* combine with red cells and agglutinate them is not impaired, although the heated virus will not elute from the cells. The agglutinating activity of heated virus is prevented by high dilutions of inhibitors such as those found in normal sera (Francis, 1947). Heating has not destroyed the capacity to combine with varied mucoproteins but has eliminated the enzymatic action which permits the splitting of the mucoprotein inhibitor in the serum or on the receptor sites of the erythrocyte. The material, which some have termed α inhibitor, migrates with the alpha globulins in electrophoresis (Tyrrell, 1954). This phenomenon is considered in detail in Chapter 4 Another inhibitor in serum is heat labile, neutralizes infectious virus and appears to be part of the natural defense mechanism This was studied by Ginsberg and Horsfall (1949) and corresponds to the labile inhibitor initially observed by Hirst (1942b)

When influenza virus and immune serum against it are mixed and red cells are then added, hemagglutination is specifically pre*vented by antibody (Hirst, 1942b)*. The immune serum can be diluted to an extent proportional to its virus-neutralizing titer before its capacity to inhibit hemagglutination by a constant amount of virus is exceeded This is the basis for the hemagglutination-inhibition test for antibodies which is described in Chapter 10. But it has also provided a most valuable set of reagents for detailed study of virus behavior, antigenic constitution and *im*munity

Complement is fixed by the interaction between virus particles and immune serum. The complement-fixing viral antigen exhibits a significant strain or group specificity (Friedewald, 1943). In addition there is a "soluble" antigen of much smaller size, 10 mμ (Lennette and Horsfall, 1940), first so designated by Hoyle and Fairbrother (1937) in preparations from infected mouse lungs The material

strains and give cross-reactions detectable by hemagglutination or neutralization tests The dominant antigen of one strain can be detected as a lesser antigen of another On this basis the hypothesis is advanced that strains of a given type comprise essentially the same antigens in limited number and that strain variation represents rearrangement of these antigens quantitatively or spatially (Magill and Francis, 1938, Smith and Andrewes, 1938, Francis, 1952, 1955, Salk, 1952) Strains prevalent in certain years will thus exhibit relationships with those of other times The bulk of evidence obtained by antigenic analysis of virus strains—especially by antibody absorption and multiple comparison—supports this view (Jensen and Francis, 1953, Isaacs et al, 1954) Limited comparisons by testing strains against single prototype sera provide an inadequate measure of strain relationships Studies of antibody patterns in the human population also emphasize the antigenic overlapping Younger persons exhibit antibody directed against the most prominent antigens of the strains they have encountered and retain their susceptibility to components which may dominate other antigenic complexes but have not been well represented in the antibody response to earlier strains With advancing age a broader immunity is reflected in the multivalent antibody acquired by contact with numerous primary and secondary antigens in strains encountered through the years (Davenport et al, 1953) If a ferret has been infected successively by 3 distinctive strains of Type A, absorption of the final serum with the initial strain will remove antibody to all while absorption with the 2nd or the 3rd infecting strain removes primarily antibody to the homologous strain and some of the heterologous antibody (Jensen et al, 1956) Essentially all Type A antibody can be removed from human serum by absorption with the strain prevalent during the childhood of different age groups These data emphasize the sharing of antigens between strains of different periods

Another view of antigenic variation has been that during the prevalence of a strain the population becomes immunized to its characteristic antigen so as to limit that strain's propagation, this dominant antigen is then eliminated in succeeding strains and replaced by an entirely new antigenic component (Andrewes, 1950, 1957) There is, according to this thesis, an orderly progression of variation by antigenic replacement which propels the pattern of mutation in one direction It tends to ignore the relationships which have been clearly demonstrated even between major variants of a type, as between swine influenza virus and the PR8 strain of 1934, between PR8 and the FM1 (A-prime) strain of 1947, and apparently between the A-prime strains of 1956-57 and the Asian variant of 1957 Gamma globulin prepared in 1943 had significant titers against the A-prime strains of 1947 and later, it also contained antibody to Type B strains of 1945 and 1952 (Davenport et al, 1953) Recent evidence that persons of more than 40 years of age, particularly over 70 years, display antibody to the Asian strains of 1957 (Mulder and Masurel, 1958) illustrates the return to primary position in current strains of antigens which had dominated strains of many years previous and in the meantime had probably persisted as secondary antigens Unpublished data by investigators in other countries have noted this occurrence of antibodies to Asian strains Autre temps, meme chose In opposition to the thesis of orderly and progressive replacement is the evidence that among groups of Type A strains in two successive 10 year periods, antigenic variation was a random affair rather than a chronological progression (Jensen and Peterson, 1957)

While it is currently believed that the nucleoprotein, type-specific, antigen carries the genetic determinants of the virus, the antigens represented in the hemagglutinin appear to play a dominant role in serologic and immunologic variation Variation has been observed to occur under different laboratory conditions of passage in mice, eggs, or tissue culture and on partial neutralization with immune serum in chick embryos Antigenic variants of the PR8 virus were obtained by Gerber et al (1956) by serial passage in the lungs of mice immunized with the homologous agent The variants exhibited a progressively decreasing reactivity with the parent PR8 antiserum but retained the capacity to induce antibody to PR8

Variation can be induced experimentally by recombination in which virus particles of

bined with the lipid and carbohydrate. Thus, the virus particle is pictured as developing "by fragmentation of the cell cytoplasm and that it consisted of a closely packed aggregate of soluble antigen and hemagglutinin enclosed in a membrane derived from the cell wall The chemical results would suggest that this membrane is composed of lipoprotein and mucoprotein *Treatment with ether denatures* the lipoprotein and disrupts the particle" Much of the lipid remains in the ether fraction (Frisch-Niggemeyer and Hoyle, 1956) *It is suggested that the RNA of the soluble* antigen is the fundamental replicating nucleoprotein of influenza virus Studies with P^{32} indicated that the virus nucleoprotein rapidly becomes associated with the cell nucleus (*Hoyle and Frisch-Niggemeyer, 1955*) This may be in keeping with the observations, using fluorescent antibody, that virus staining first appeared in cell nuclei due to the presence of soluble antigen (Watson and Coons, 1954, Liu, 1955) The structure of *fully formed virus is considered to* be a central core of type-specific nucleoprotein surrounded by strain-specific mucoprotein bound together by lipoprotein and mucoprotein, possibly derived directly from the cell These components may correspond to the inner body, the dense membrane and diffuse outer coat of active virus particles revealed by electronmicroscopy (Morgan et al, 1956) Serologic evidence strongly indicates that the dominant antigen is superficially located. It is not known which components are the essential factors in endowing virus with infectivity, but evidence from many sources suggests that this is acquired at or near the time of release through the cell surface Ada and Perry (1956) found no consistently proportionate relation between infectivity and nucleoprotein, but the possibility exists that this is an important factor in virus maturation It may be that large inocula, including inactive virus, use up the mucoprotein substrate of the cell necessary for this transformation, even as sulfonic acid prevents the release of influenza virus from infected cells (Ackermann and Maassab, 1954c). Although the failure of developing virus to gain completion *has not yet been explained* satisfactorily, the fact that the various reacting materials can be identified serologically as independent components makes easier consideration of the idea that their production may be proceeding in cells without attaining full viral configuration or, conversely, resulting from disruption of larger structural *units—including inactivated* virus

Serologic behavior of influenza virus can be related to the identified components *Complement-fixing type-specific antigen is a* ribonucleoprotein common to strains of Type A but differing from that of Type B. This material represents the soluble antigen and seems also to be the type-specific complement-fixing antigen in whole virus particles The hemagglutinin, a mucoprotein, exhibits strain-specific behavior which is characteristically inhibited by immune serum *It* is antigenic and induces protection of susceptible animals comparable with that attained with the virus *particles themselves* Antiserum to this fraction contains neutralizing antibodies The hemagglutinating component appears also to be the strain-specific complement-fixing anti*gen of the virus particle. The hemagglutinat*ing antigen can be absorbed by red cells from a preparation of disrupted virus, leaving the soluble antigen in relatively pure state (Frisch-*Niggemeyer and Hoyle, 1956*).

Within the *2* most studied types of influenza virus, A and B, numerous strains have been found to differ from one another serologically and immunologically, while retaining demonstrable similarities The variants within Type A strains, for example, tend to fall into subgroups or families This subject was reviewed by Jensen (1957) By selective procedures some difference can be detected between almost any 2 strains, indicating that serologic variation is a continuous phenomenon Vaccination of man has provided major information as to the immunologic significance of variants which have come into distribution. The differences are most distinct when sera prepared by single injection of nonsusceptible animals are employed in cross-hemagglutination or neutralization tests The similarities are demonstrated best in serum of man or other susceptible animals recovering from infection, or after hyperimmunization of susceptible or refractory species The results indicate that in addition to a common soluble antigen, individual strains contain a number of antigens which exist also in other

true when illness of that type represents a distinct change from the character of illness which has previously been occurring in institutional or other congregations continuously under observation. There is no clinical basis at present to differentiate influenzas A, B, C and D. Since the onset of numerous illnesses may superficially resemble influenza, and numerous transient illnesses pass by without clinical need for etiologic diagnosis, danger exists that the diagnosis of influenza will be made as a substitute for more exacting differentiation. Except in special circumstances, the effort and the expense required for laboratory confirmation is scarcely warranted in the sporadic suspect. However, when cases occur en masse, further study is recommended; there are at present numerous facilities for assistance which should be called upon. Details of laboratory diagnostic procedures are given in Chapter 10 by Jensen (1956) and by Lepine (1954).

The two procedures of most specific diagnostic worth are the isolation of virus from the patient and the demonstration of antibody rise in the serum of the patient to a type of influenza virus. Isolation of virus from throat washings of the acutely ill patient is the most rapid procedure. Optimally, the material should be obtained in the first 3 days of illness by having the patient gargle thoroughly and repeatedly with small amounts of broth, distilled water, or skimmed milk to a total volume of 15 to 20 ml. The gargling are collected in a clean stoppered container and taken to the laboratory where they are promptly injected, after addition of penicillin and streptomycin, into the amniotic sac of 10- to 14-day embryonated chicken eggs (Burnet, 1940) or frozen at −72° C. Nasal washings can be used as well. The fluids are harvested after 2 to 4 days of incubation and tested by hemagglutination for presence of virus. If present, virus can also be identified promptly by appropriate immune serum, and thus an unequivocal diagnosis may be established promptly. The percentage of virus isolations varies from one outbreak to another and with technical competence, but 80 per cent recovery is not unusual. Allantoic inoculation is easier but less sensitive, although Type B strains may be recovered with equal frequency by this method. After initial isolation in amniotic sac, most strains of Types A and B can be passed satisfactorily by the allantoic route, but Type C strains require amniotic transfer. Variation in the agglutination reaction with cells of different species may also be of presumptive value in identification as follows:

Virus Type	4° C Cells		22° C Cells	
	Chick	Guinea Pig	Chick	Guinea Pig
A	±	+	±	+
B	+	+	+	+
C	+	−	−	−
D	+	+	+	+

Human O cells are commonly used and behave similarly to guinea pig cells.

Isolation of virus by intranasal inoculation of ferrets is also highly efficient with strains encountered during well-marked epidemics of influenza A, but in lesser prevalences the clinical manifestations induced in the ferret may be irregular. Virus can commonly be recovered from the turbinates and transferred to eggs. Serologic tests with sera of the recovered ferret will confirm the nature of the virus infection, even though clinical signs are absent. Type B strains are decidedly less pathogenic for the ferret than those of Type A. Type C strains produce infection essentially localized in the turbinates without significant febrile reaction. Isolation of Type D virus in ferrets has not been reported.

Influenza virus can be transmitted directly from human throat washings to mice and hamsters, but its adaptation is slow. Type-C virus is not readily established in mice, although its isolation in that species has been reported (Fukumi et al., 1951).

The use of tissue culture in virus isolation

tion of Type A virus (Vogel and Shelekov, 1957).

Various authors have reported the value of cytologic studies of respiratory excretions from patients for influenzal diagnosis, the presence of injured epithelial cells is said to be diagnostic. Similarly, examination of scrapings from the trachea of the infected chick embryo has been used. The use of fluorescent

2 different strains effect combination of distinguishing properties of one strain with others of a second strain (Burnet and Lind, 1951) Gotlieb and Hirst (1954) have shown the derivation of serologically mixed strains after double infection of embryonate eggs Similar results have been obtained by using ultraviolet irradiated virus of one strain and active virus of another (Baron and Jensen, 1955). The development of this field can be of great theoretical and practical interest (see Jensen, 1957)

Variation in the avidity with which strains are neutralized by antibody is observed Van der Veen and Mulder (1950) have classified them in P, Q and R phases according to their behavior in the hemagglutination-inhibition reaction with convalescent ferret serum The P-phase virus is inhibited to high titer by its homologous serum only Q-phase virus is poorly neutralized even by homologous serum, while the R phase is inhibited to high titer by homologous sera and by sera against heterologous but related strains They may be altered by passage in mice or in eggs, but the explanation of their behavior is not well understood It may be related to location of antigens (Isaacs et al, 1954), the content of primary and secondary antigens, or even to the content of lipid components which may interfere with serologic reactions Newly isolated strains are commonly in P phase, but distinct exceptions are encountered and Q-phase behavior is encountered not infrequently Considerable differences exist in the extent to which hemagglutination by strains is prevented by nonspecific inhibitors in sera This is encountered with newly isolated strains and can cause confusion in serologic identification, especially if the strains are poorly neutralized by specific antibody.

Newly isolated strains often agglutinate guinea pig erythrocytes better than those of the chicken, but after passage in eggs this tendency may disappear, O-D variation (Burnet and Bull, 1943). Type B strains show this effect less than strains of Type A. Type C strains do not agglutinate guinea pig cells and act on chicken or human O cells best at refrigerator temperature (Taylor, 1951; Minuse et al, 1954) According to Hirst (1950), they act upon a receptor site on the erythrocytes' surface differently from that of other members of the species The Sendai Type D virus adsorbs and elutes rapidly from erythrocytes so that it may be necessary to read hemagglutination tests in 30 minutes.

Strains differ in their virulence and adaptability to experimental animals Certainly epidemic distribution and virulence are not necessary accompaniments, since a mild strain may be widely dispersed Alteration in antigenic composition may be an important asset but is not necessarily associated with high virulence Epidemic spread is undoubtedly related to enhanced affinity for susceptible cells, one may ask whether an exaggerated tendency to combine with nonspecific inhibitors may reflect the enhanced affinity for mucoproteins of the respiratory tissues and thus identify the epidemic character. The epidemic strain, well adapted to man, probably requires a smaller inoculum and has a more rapid growth curve than strains of limited extension, as is seen among strains adapted to new species experimentally (Wang, 1948, Davenport and Francis, 1951)

Influenza virus infection has a distinct influence in promoting bacterial invasion In experimental animals, pre-existing bacterial infection is activated to produce more severe disease. *Introduction of bacteria and virus* simultaneously is less disturbing except with swine influenza But introduction of organisms of low virulence after establishment of virus infection enhances their virulence as shown by subsequently retained characteristics (de Torregrose and Francis, 1941) The association between virus and staphylococcus in severe human disease is of interest because each exerts a necrotizing action The invasion of the virus-damaged tissue by staphylococci, producing deep tissue destruction, has been likened to wound infection

DIAGNOSIS

The diagnosis of influenza can be suspected on clinical grounds by exclusion or by careful observation of the individual case. When acute febrile illnesses of abrupt onset and 3 to 4 days' duration begin to occur in rapid succession without signs or symptoms representative of other recognizable disease, influenza should be suspected This is particularly

true when illness of that type represents a distinct change from the character of illness which has previously been occurring in institutional or other congregations continuously under observation. There is no clinical basis at present to differentiate influenzas A, B, C and D. Since the onset of numerous illnesses may superficially resemble influenza, and numerous transient illnesses pass by without clinical need for etiologic diagnosis, danger exists that the diagnosis of influenza will be made as a substitute for more exacting differentiation. Except in special circumstances, the effort and the expense required for laboratory confirmation is scarcely warranted in the sporadic suspect. However, when cases occur en masse, further study is recommended; there are at present numerous facilities for assistance which should be called upon. Details of laboratory diagnostic procedures are given in Chapter 10 by Jensen (1956) and by Lepine (1954).

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The use of tissue culture in virus isolation has been studied inadequately but is worthy of more consideration. Viruses of various types can be recovered by such procedures. Recently, studies of virus isolation in cultures of monkey kidney cells, using added guinea pig erythrocytes as indicator, have been reported to yield efficient results and rapid identification of Type A virus (Vogel and Shelekov, 1957).

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antibody to detect virus in nasal secretions has been proposed (Liu, 1955a). Detection of hemagglutinins in throat washings or assay of normal inhibitors has also been studied as means of rapid diagnosis but has not been found to be generally suitable.

Serologic diagnosis is based on the fact that recovery from influenza virus infection is accompanied by the development of antibodies to the virus They may be detected 5 to 7 days after onset and reach their peak in 12 to 14 days Since a significant proportion of the population, especially adults, may possess demonstrable antibodies as a result of earlier experience with related strains, it is essential that 2 specimens of sera be obtained, one in the early acute phase and another in convalescence, for comparative titrations of antibody level. Little or no antibody may be present in the initial specimen from children or in that of older persons when strains differing sharply from those of earlier prevalences are encountered Under other circumstances moderate antibody titers may be encountered at the time of onset The convalescent level of antibody is then evaluated in relation to the initial titer. A significant rise in antibody must exceed the margin of error of the technical procedure The methods in general use are reasonably accurate within a 2-fold variation A rise from no demonstrable antibody in the lowest dilution of serum to an extent that 2 or more higher dilutions exhibit an antibody effect against a specific type of influenza virus or strain can, therefore, be considered significant In the presence of antibody initially present, a specific 4-fold rise is usually acceptable Commonly, the increases are greater.

may be noted in persons recently vaccinated whose sera are tested with the vaccinating strains. However, when tested against the epidemic strain, the titer of the first-stage serum may be found to be considerably lower, and then a diagnostic increase is observed. In other instances the titer may be high because the true onset was earlier than that recorded, and viral antibody has developed before secondary complications began. If results of this nature are obtained in the hemagglutination-inhibition reaction, the presence of nonspecific inhibitor should be suspected, and then the sera should be treated to eliminate that effect and retested.

The serologic procedure most commonly employed is that of hemagglutination-inhibition in which antibody content is measured by a specific capacity of serum to prevent agglutination of erythrocytes by the test virus (Hirst, 1942b). Chicken cells, human O cells, guinea pig cells are used most frequently, although those from other species may be employed in special circumstances The patient's sera should be tested against the different types of influenza virus unless the epidemic strain is available. Antibody rises of 4-fold or greater are obtained with homologous strains and frequently with closely related strains of the same type. Ordinarily, an increase to other types is not observed. With strains newly isolated in eggs the convalescent serum titers may be low, and the virus may be especially susceptible to normal inhibitors This creates difficulty in obtaining satisfactory readings with low dilutions of serum, and the impression is gained that no antibody rise is occurring After further egg passage or after mouse passage, their serologic behavior may be improved

quired antibody to Type A strains by previous exposure will ordinarily exhibit in convalescence a rise to other related strains in addition to that of immediate infection In fact, the convalescent titers to other strains may reach new levels well above those to the homologous strain, although the increment of increase to the latter is much higher These effects emphasize the joint relationships between strains and their influence as secondary stimuli to enhancement of previously acquired antibody. Their significance is seen in that the younger segments of the population behave more as immunologic virgins with relatively low convalescent titers limited to recently dominant antigens, while adults present a response which may be detected with varied strains, depending upon age and experience. The effect of previous experience may also be reflected to some extent in the response measured by complement-fixation test. However, when epidemic strains arise which are sharply different antigenically from those of recent years, the hemagglutination-inhibiting response may appear to be highly strain-specific

through much of the age range. Nevertheless, even in 1957 some cross-reactions with earlier strains have been observed in convalescence.

The complement-fixation test with soluble antigen is type specific, essentially unaffected by nonspecific inhibitors and may be conducted with antigen from any of a number of strains of the same type. Vaccination ordinarily induces limited complement-fixing antibody so that infection in vaccinated persons can be detected without interference from pre-existing high hemagglutinin inhibition titers to other strains. Using allantoic fluid as viral antigen, a greater degree of strain specificity may be encountered, but this is not usually a serious limitation. When using progressive dilutions of serum, a 4-fold or greater rise is significant. Good responses are also obtained with antigen prepared from infected mouse lungs.

The mouse protection test for neutralizing antibodies is comparatively precise. The titers obtained are roughly proportionate to those of the hemagglutination-inhibition test but numerically may be quite different. There is less strain specificity, and protective antibody may be demonstrated to strains which appear quite different in the agglutination test.

The clinical differentiation of influenza from other illnesses may depend upon the absence of characteristic features of those illnesses. The typical common cold is more an afebrile illness with profuse coryza and congestion of the upper respiratory tract predominating. Acute streptococcal pharyngitis exhibits the localized pharyngeal inflammation with exudate and leukocytosis. Coxsackie virus infections may present herpangina, pleurodynia or aseptic meningitis. Acute respiratory disease associated with adenovirus infection is difficult to differentiate, although the course may be longer, and in some outbreaks the frequency of conjunctivitis is a distinctive feature. In military populations the selective distribution of adenovirus illness among new recruits is different from influenza which commonly affects all groups. In the incipient stage, particularly, many other illnesses, such as psittacosis, Rift Valley fever, lymphocytic choriomeningitis, ECHO virus infections, bacterial pneumonia, or sinusitis may resemble influenza. However, adequate etiologic differentiation remains primarily a matter of laboratory investigation.

TREATMENT

There is no specific therapy for influenza. In the usual uncomplicated case of influenza, treatment is essentially palliative and symptomatic. The most important measure is the assurance of complete bed rest. It is better for the patient to remain at home without visitors than to be hospitalized where exposure to bacterial infections may be extensive. The room should be kept at a comfortable temperature; cold air aggravates coughing and irritates an inflamed respiratory tract. A simple diet with adequate fluids is well tolerated. Simple medications will relieve the myalgias; heavy sedation should be avoided. If cough is troublesome, cough syrups such as elixir of terpine hydrate with or without codeine are helpful. Codeine is useful for general relief as well, since it provides comfort without unduly obscuring the febrile course. Acetylsalicylic acid or similar antipyretics give great relief, but the perspiration which accompanies their effect may be disturbing. Substernal soreness may be relieved by use of hot-water bottle or electric pad. Bland gargles may relieve pharyngeal discomfort. Nose drops or inhalants are satisfactory for nasal congestion, but oily sprays should be avoided.

Sulfonamides and antibiotics do not affect the virus infection. However, there is a great tendency to use them under the guise of preventing complications. The council on Drugs of the American Medical Association has recently issued the following statement prepared by a Special Committee on Influenza (1957):

> Antibacterial compounds such as sulfonamides or antibiotics should not be generally used in the prophylaxis of bacterial infection in patients with influenza. The prime reason for this recommendation is to prevent the development of disease-producing strains of micro-organisms which would be resistant to sulfonamide or antibiotic therapy. The secondary reason is to obviate the development of reactions of sensitivity or of direct toxicity to antibiotics or sulfonamides. The possible exceptions to this injunction are (a) pregnant women, debilitated infants, and older individuals, (b) patients being treated for other bacterial infections with sulfonamides or antibiotics who develop influenza, and (c) patients

suffering from chronic, nonallergic respiratory tract disease.

All patients ill with influenza in whom it has been demonstrated that secondary bacterial infections have developed should be treated with a sulfonamide or antibiotic of choice. In general, the following recommendations can be made as to the choice of sulfonamides and antibiotics. (a) pneumococcic pneumonia: penicillin, broad-spectrum antibiotic, sulfonamide,

monia.

sulfonan

monia· broad-spectrum antibiotic, (d) micrococcic pneumonia· this will be a real problem because of the resistance of strains of these organisms to sulfonamides and antibiotics Careful testing of the sensitivity to the various antibiotics of micrococci isolated from patients ill with pneumonia should be done and the choice of the antibiotic made upon data garnered from these tests If the condition of the patient is so grave as to necessitate immediate therapy, massive intravenous doses of penicillin plus 1 Gm of streptomycin per day may be administered while the results of antibiotic sensitivity tests are being awaited

Chloramphenicol appears to have greater efficacy against *H influenzae* than many of the other compounds

Since in large scale epidemics medical attention may be limited, the patient or his attendants should be informed that if symptoms increase in severity, fever persists beyond 3 or 4 days, or other serious change, the physician be notified, he in turn should be alert to possible complications In the small number of fulminating cases emergency measures must be applied. Since they usually exhibit acute pulmonary edema, oxygen and carbon-dioxide exchange is reduced, and treatment should be directed to its relief. Sedation must be used with caution Patients with mitral stenosis, chronic pulmonary disease, and pregnant women appear to present special risks apart from the hazards of bacterial infection. In the presence of concomitant staphylococcal infection the use of adrenal cortical preparations may be considered.

Some use has been made of animal immune serum or human convalescent serum without very gratifying results However, Russian investigators have continued in the use of intranasal insufflation of serum for treatment, but the evidence of its effect is not convincing Vaccination has no place in treatment.

Patients should return to normal activity with care, particularly if this involves strenuous physical activity. Adults commonly experience weakness and slow return to full strength. In a proportion of cases mental depression and signs of neurasthenia may be very troublesome. This requires sympathetic but confident support by the physician.

EPIDEMIOLOGY

Because the clinical manifestations of influenza are not of themselves sufficiently unique to identify the individual case or small group of cases adequately, influenza has been predominantly an epidemiologic diagnosis The classic features of influenza are its occurrence in epidemics which arise abruptly and spread rapidly but irregularly over a region. The disease appears in small, focal outbreaks, in epidemic or pandemic form Outbreaks are most common in the period from early autumn to late spring but may begin or extend during the warm seasons. In an area the peak may be reached in 3 weeks, and the course essentially completed in another 3 to 4 weeks There is a high morbidity and low mortality, although the occurrence is usually reflected in an excess incidence and mortality from pneumonia and in an increase of total mortality built up from increased deaths of persons with chronic, debilitating disease Despite the absence of pathognomonic

abrupt onset with chills, aches, fever, prostration without prominent respiratory signs, and uncomplicated recovery beginning after 3 to 4 days of illness Influenza can be considered

viruses which may differ qualitatively and quantitatively but possess similar pathogenic properties Variations in severity and extent can be related to differences in the host population, in the primary causative agent and in environmental influences This concept of the epidemiology of influenza permits its consideration according to principles underlying the ebb and flow of other epidemic diseases of man transmitted by the respiratory route.

Morbidity

Incidences of 20 to 40 per cent have been noted repeatedly in general populations during extensive outbreaks. In isolated populations or in concentrated groups even higher attack rates may occur. Based on house-to-house surveys in one area, Collins (1944) reported a civilian attack rate of 15 per cent during the 1943-44 epidemic of influenza A. In certain military establishments the combined incidence of hospitalized and dispensary cases was 15 to 16 per cent, and in 1947 it was 8 per cent. In the 1957 pandemic extremely high rates existed in various countries, adequate data in the United States are not yet available. At carefully observed military posts rates as high as 10 per cent occurred, while on naval vessels rates of 50 to 60 per cent have been reported. In outbreaks of intervening years, however, rates of 2 to 5 per cent have been more frequent. Outbreaks of influenza B have also varied. A population survey of influenza B in early 1937 indicated an incidence as high as 30 to 40 per cent in some affected communities, while in 1954-55 in a closely observed population it was about 2 per cent. In addition to clinical disease, a high frequency of inapparent infection can be detected serologically.

Age Distribution

The age distribution of cases is best determined from specific surveys. These data emphasize that the highest incidence is usually at 5 to 9 years. There is a sharp reduction in the 15-to-24 age group followed by a rise between 25 and 34. Thereafter a downward trend follows to a further low in older ages (Collins, 1944, Francis 1953). A somewhat similar distribution was observed with influenza B in 1936 (Francis, 1937). On the basis of current data the 1957 pandemic exhibited an age incidence of this form also. Preliminary information from the U. S. National Health Survey (1958) has provided weekly rates of new bed cases in the United States of 28 per 1,000 at 0-5, 40 at 5-19, 16 between 20 and 64, and 9 in persons over 65 years of age.

Mortality

There is little information regarding fatalities from the viral disease alone. The ascribed mortality predominantly relates to pneumonia largely associated with bacterial invaders. The mortality rate is a resultant of attack rate and case fatality. In the majority of epidemics case fatality scarcely exceeds 1 per 10,000. However, it is estimated that in the autumn of 1918 the average case fatality in the affected population of Europe and North America was about 2 per cent (Jordan, 1927). In certain native or inexperienced populations mortality rates of 10 to 20 per cent were reported. Among Allied military forces in France in 1918 case fatalities of 6 to 7 per cent were recorded from influenza and pneumonia. Great variation occurred in different military installations in the United States with reported case fatalities from 2 to 10 per cent. It is apparent, then, that mortality varies with the extensiveness and the severity of the epidemic. The latter is influenced by the virulence of the virus and the frequency of bacterial complications. For instance, the influenza A of 1943-44 in the United States had the highest incidence since 1918, but the lowest recorded percentage of cases complicated by pneumonia, nevertheless, by virtue of the high incidence the resultant excess mortality was high (Collins, 1944). In 1957 again the mortality was low everywhere despite extremely high incidence. The age distributions of mortality and case fatality differ significantly from that of age incidence. Ordinarily, the age mortality curve from respiratory disease has a skewed U shape, proceeding from a high point in the first years of life to the lowest period at 15 to 25 years and increasing progressively thereafter to its highest point in old age. Influenza tends to displace this mortality curve upward with increased rates beginning at earlier ages. The lowest case fatality ordinarily occurs in the age period of highest influenza incidence, while it tends to become exaggerated in the period above 40 years where the incidence is decreasing. In 1918, however, the frequency of reported pneumonia was high at all ages, but strikingly the age group of 20 to 40 exhibited the highest incidence of complicating pneumonia with a resulting high case fatality which distorted the over-all age mortality curve to the form of a W. The pneumonia rates declined consistently after 40, although case fatality increased to the peak after 60. This deviation with high mortality in middle age has been said to indicate a different etiologic and epidemiologic entity. It may more reasonably be attributable to an exaggerated physiologic stress of pneumonia on males of this age, for it was distinctly less evident in females. The factors responsible for the increased case fatality of 1918 appear to differ from those responsible for influenza morbidity. The increased immunity of older ages to the vi-

rus infection made them less susceptible to pneumonia, but when they developed pneumonia their fatality rates were higher (Francis, 1953). Stuart-Harris (1953) has called attention to the increasing proportion in recent years of all deaths related to influenza which has been observed in persons over 55 years of age. The excess mortality from all causes which occurs during influenza epidemics also tend ... young ... other ... ion A recent review by Collins and Lehmann (1957) emphasizes the progressive and the steep decline in mortality from influenza and pneumonia since 1936-37 and the advent of effective therapeutic drugs. Moreover, the excess mortality in epidemic periods has also been declining from 44.4 per 100,000 in 1928-29, 18.4 in 1936-37, 14.4 in 1943-44 to 6.9 in 1953. From 1953 to 1957 essentially no excess mortality was detected.

Epidemic Spread

Procedures for the identification of influenza viruses have provided accurate means for mapping the development and the spread of recurrent outbreaks on a broad scale. Nevertheless, this information could be correlated only when information from active, regional laboratories was pooled. A series of detection centers was established for this purpose by the Commission on Influenza in 1942 and augmented by laboratories of American Armed Forces in various regions. The World Health Organization began in 1947 the development of a co-ordinated system which is now world-wide. Continuous observation has demonstrated that extensive outbreaks may be preceded for some months by small, apparently local, flurries caused by the same strain of virus. This was first noted for influenza A in 1943 (Hare et al., 1943; Francis, 1945). In 1945 scattered prevalences of influenza B were identified for 8 months in the United States, in the Pacific area and the Southern Hemisphere before the major peak in November of that year occurred in North America and Europe (Francis et al., 1946). In other years, a sampling of illness has commonly failed to reveal influenza until the beginning of a definite outbreak; however, these observations have usually involved only large metropolitan centers and few laboratories. It has not been uncommon to find that the strain in circulation during the winter months in the Southern Hemisphere then appears 6 months later during the cold season of the Northern Hemisphere or vice versa. Areas such as Puerto Rico and Hawaii with little seasonal variation appear to behave as crossroads. From July to September, 1940, influenza A was prevalent in the Southern Hemisphere, the West Indies and Hawaii; it reached the West Coast of North America and spread eastward during that winter (Francis, 1943). The A-prime strain (Cam) which was isolated in Australia in 1946 was found to be a forerunner of the initial A-prime dispersion in the Northern Hemisphere early in 1947; they were first recognized in Russia in 1949 (Smorodintsev and Zhdanov, 1957). Chu et al. (1950) described the northerly spread of an A-prime outbreak through Europe in 1949. The best studied example of antigenic uniformity in a pandemic distribution is that of 1957. Although variations in serologic reactivity were noted, antigenically the Asian strains were essentially identical wherever isolated. Early in 1957 influenza in the United States and other parts of the world was caused by A-prime strains, in the fall, Asian strains prevailed. However, successive waves in a region may be related to different types of virus. In the early months of 1940 influenza B was prevalent, but influenza A occurred in the autumn; influenza A may be prominent in one year and influenza B in the next. On the basis of limited experience it was suggested that influenza A had a 2- to 3-year cycle, while that of influenza B was 4 to 5 years, but this is far from regular. Since 1935 there have been 11 identified general prevalences of influenza A in the United States and 5 of influenza B (Table 28). The experience of other countries is similar, and there is reasonable agreement in the timing of the strain prevalences in different countries. Interestingly, the antigenic character of the strains identified in different parts of the world at a given time is much the same. This is particularly true when major prevalences occur. There are repeated indications that under certain circumstances strains of different character may be encountered in a given area as first noted in 1936 (Smith and Andrewes, 1938). Isaacs and Andrewes (1951) have described the experience in Great Britain in 1950-51 where they conclude that a confluence of somewhat different A-prime strains occurred, one derived from Scandinavia, while the other, which caused more severe disease in Liverpool, came up from the South. Scattered cases of influenza B have been noted interspersed with epidemics of influenza A; influenza C has been detected in

conjunction with outbreaks of influenza A or B. The outbreak of influenza D in Vladivostok occurred in company with influenza A (Gerngross, 1957). Double infections also occur. Therefore, mixed prevalence may be expected, although the tendency toward conformity in strains from a widespread epidemic is dominant despite demonstrable but limited individual variations

These observations indicate that the strains of virus are transmitted widely and readily with mutants of epidemic capacity dispersed in multiple foci until a sufficient concentration of infection builds up or environmental conditions, such as cold weather and increased crowding of the population, enhances opportunity for transmission These considerations are of importance in interpreting the source and the distribution of epidemics

Origin of Epidemics

No reservoir of human influenza virus has been demonstrated such as the lung worm indicated by the work of Shope (1941) for swine influenza virus in the United States As mentioned elsewhere, there are reports of recovery of strains corresponding to human virus from other animals (cf Host Range)

In the earlier literature repeated suggestions are made that major epidemics arose from endemic sources in Turkestan or Eastern Russia and extended to the West along the routes of travel (Jordan, 1927, p 74) Evidence indicates that while the initial source of the virus is not known, the severe pandemic strain of 1918 was primarily recognized in relation to the military areas of France. Thereafter spread was rapid and diffuse. Nevertheless, opinions that it was of Asian origin were commonly expressed There is not at present sufficient information to assume that a single localized focus or reservoir exists in which epidemic variants primarily arise The experiences of 1943 and 1947 influenza A and 1954-55 influenza B readily suggest that each of those variants arose in a different place Whatever the source, there is no doubt that spread is essentially a point-to-point phenomenon, and numerous small foci may be established before coalescence into a full-blown epidemic occurs

The spread of the 1957 epidemic provides striking illustrations of the mode of dissemination From the interior of China in late February and March the disease extended in early April among refugees in Hong Kong Extension to other Pacific areas was commonly and clearly related to specific imports by ship or plane American naval vessels touching [illegible] of [illegible] it [illegible] at [illegible] vessel off Newport, R I., was noted in May Then an outburst occurred in a student conference at Davis, Calif , members of that conference introduced it into other regions, including another conference in Grinnell, Iowa From that, secondary distributions occurred widely California representatives took the disease to an International Boy Scout

Table 28 Identified Influenza Prevalences in U.S.A Since 1930

Swine A	A	A-prime	Asian	B	C
1930-31	(1) 1932-33				
	(2) 1934-35				
	1936-37			1936 early	
	1938-39				
	1940-41			1940 early	
	1943-44				
				1945 early and prolonged	
		1947 early			+
		1950 early			+
		1951 early			
				1952 early	±
		1953 early			±1954-55
				1955 early	
		1957 early	1957		

Jamboree, whence further scattered foci were established Other outbreaks were related to migrant laborers from the Southwest. In the meantime, the epidemic had spread, often in limited extent, to other areas radiating from the Asian focus so that by mid-June influenza had circled the globe It was notable that initial prevalences in many areas were seen in crowded populations or special groups in close quarters The peak of prevalence occurred in the last 2 weeks of October, and it is estimated that in the United States there were over 11 million new bed cases in each of those weeks (U. S National Health Survey, 1958). Thereafter, a progressive decline occurred, although scattered illness persists Were it not for the close attention and the rapid communication of information, this outbreak, too, could have given the impression of rising spontaneously in numerous parts of the world (Figs 109 and 110). However, the

of the virus by association but also demonstrates that a sufficient nucleus of infection is needed to induce major epidemics This is undoubtedly increased by a sizeable proportion of inapparent infections. Another effect of crowding is seen in the extremely high incidence in congested populations of the East, in the companies of ships and certain industrial groups This was further observed when students convened in schools and colleges in the early autumn, the disease began promptly and led to high incidence among them The impression was gained that it was frequently more marked when this represented a close mingling of students from many areas rather than merely local aggregations

One concept of the development of a pandemic is that it erupts simultaneously in many areas This implies that virus possessing essentially the same characteristics arises independently in many different areas—presumably because of a variation forced by immunity of the population It is difficult to accept this in view of the comparative antigenic uniformity of the strain in wide circulation It is more likely that the early distribution is unrecognized

The emergence of a new epidemic strain [illegible]

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sources of the individual host in which it arises

and that of the surrounding population Its ability to disseminate is, then, determined by its infectious property, that is, its capacity to multiply in a new host to which it gains entrance. That capacity may be progressively enhanced, as is seen in the experimental adaptation of a strain to a new species so that the amount of inoculum required is decreased, the lag period is eliminated, more virus is produced, and severity of injury to the host may be increased. Thus, pathogenicity and dispersibility may be established and stabilized at a high level with passage through the human population Virulence or severity of injury may also become stabilized at a mild or severe level but is a variable independent of infectiousness, except as the virulent virus depends on other properties for its transmission Theoretically, as infectivity increases, virus may survive in more resistant hosts and adaptation of the strain to higher virulence may ensue. However, it is the common experience that when a strain becomes sufficiently well adapted by passage in a human population as to extend widely, its pattern of behavior is well established. In view of the high degree of mutability among influenza viruses, there must be countless variants of influenza virus which appear and promptly disappear because of lack of appropriate infectivity or opportunity to develop it.

Presuming that the mutant virus arises in a resistant host by reorganization of its antigenic components so that it can survive, the most favorable environment for its further propagation is the young portion of the population which will have had the least previous experience. Moreover, concentrated populations will heighten the chances of rapid passage One can believe that this can happen in a limited community but still lead to a dead end because there is no outlet to the wide world Unless some mode of maintenance besides continued transmission in man or other species is available, that strain is lost Or the virus may acquire ready infectivity but be stabilized at an immature nonpathogenic, avirulent state (Andrewes, 1950), behaving as an antigenic neutron, transmissible RNA, or an R form which can be induced subsequently to acquire more specific character. However, the evidence is clear that large epidemic prevalences are essentially limited to the distribution of a strain of matured virus which probably originated and became established initially in a limited area Occasional strains of local character or strains which have not completed their excursions in the popula-

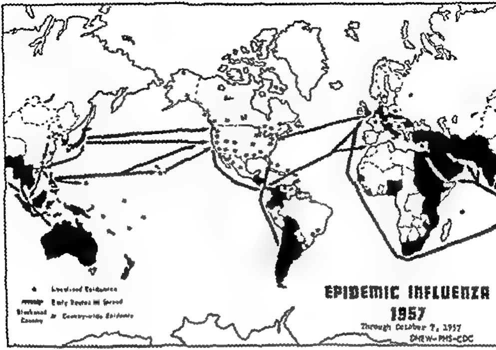

FIG 109 (U S Department of Health, Education, and Welfare, Public Health Service)

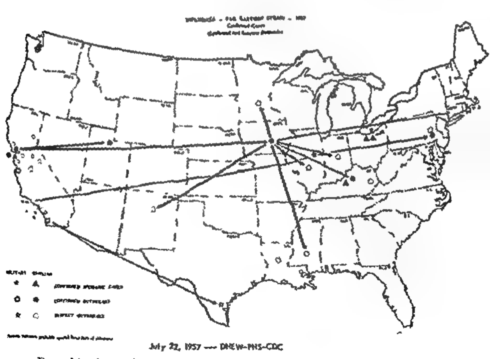

FIG 110 (U S Department of Health, Education, and Welfare, Public Health Service)

tion may well be encountered in a community That pandemic distribution is not a function of virulence and that virulence is not a requisite of sharp antigenic variation is well demonstrated in the experiences of 1947 and 1957 when marked deviations of the Type A strains were associated with pandemics of mild character Nevertheless, mild but world-wide distributions are commonly overlooked, and the term pandemic is used to denote a wide distribution of the more virulent agents. In reality it seems likely that most strains encountered in epidemic form achieve pandemic distribution

Immunity

The duration of immunity to influenza is not adequately determined, complicated as the problem is by multiple antigenic variants Shope (1931) has noted that adult hogs which have gone through one epidemic are not affected the next year, and the annual recurrences appear to be limited to the new offspring This may account for the antigenic uniformity of the swine influenza strains in America In mice, a durable immunity after infection is observed In ferrets, immunity wanes after a few months, but a modified resistance persists which prevents pulmonary involvement, although it may not prevent febrile illness and damage to the respiratory epithelium on reinoculation However, resistance can be made more effective by repeated respiratory exposures during the immune period. It is noteworthy that the repair of epithelium is markedly accelerated in the partially resistant animal and may be part of the defense mechanism

Experimental studies in man have shown that 4 months after a clinical illness induced by inhalation of Type-B influenza virus a third of the subjects exhibited illness again when resprayed with the same virus (Francis et al., 1944) Similar results were reported by Henle et al (1946) after influenza A Still other experiments by the Commission on Influenza (1943) obtained by second inhalation of Type-A virus after 2 months revealed firm resistance. Identified second bouts of naturally acquired influenza have been observed in children a year after an attack by a closely similar virus It is well recognized that adults, too, may develop influenza at

itation. Family studies have demonstrated repeatedly that those members affected in one epidemic are less likely to be involved by the next outbreak of the same type In fact, the impression from detailed histories of experiences indicates that the resistance may persist for extended periods There is no evidence of cross-immunity between influenza A and B

Epidemiologic studies have shown, however, that there is a relation between the level of circulating antibodies and resistance to either natural or induced infection Because influenza virus infection is an attack upon a superficial tissue which is essentially extravascular, the influence of circulating antibody in preventing injury to the respiratory epithelium is believed to result from the diffusion of these antibodies into the nasal secretions which at the portal of entry can combine with virus and prevent its pathologic action (Francis, 1942). They are increased after infection or after subcutaneous vaccination (Francis et al, 1943), reflecting at a lower level the enhanced titers in the blood Antibody developing after infection persists for

tected by customary procedures However, when they are re-exposed to the same or similar antigenic strains by infection or vaccination, they exhibit an accelerated and broadened antibody response to viruses of that type (Quilligan et al, 1948) Persons of different ages present different patterns of antibody, reflecting the nature of the strains to which they have been exposed during their lifetimes The sera of children contain antibodies to the dominant antigens of viruses with which they have recently been infected Since these are primary experiences, there is limited crossing with secondary antigens The older age-groups have a broader serologic activity It reflects the reaction not only to more

antibodies are first found in a population is determined by the history of influenza prevalence in the community. The frequency rises rapidly so that by 10 years of age the majority of individuals will possess antibody to recent strains part of which must certainly be antibody acquired from secondary antigens of related strains When pools of sera representing the age span of a population are tested against different strains of virus whose periods

of prevalence are known, it is seen that the highest titer exhibited by an age-group is to the strains which were circulating at the time of that group's childhood This has led to formulation of "the doctrine of original antigenic sin" which says that the antibody which characterizes an age-group through life is that to the dominant antigen of the virus present in the initial infection of life, as repeated stimuli are received from exposures to related strains this primary antibody is successively enhanced, presumably by common lesser antigens, so that it maintains the highest titer at all times Nevertheless, there is *a progressive broadening of antibody coverage* to the multiple antigens of varied strains of influenza virus, and the response to dominant antigens of later infecting strains is dampened by the composite antibody which has developed.

Epidemic Recurrences

The young segment of the population is typified by antibody to A-prime strains which appeared in *1947*, persons of *18* to *30* by antibody to typical A strains (PR8), persons of 30 to 60 by the swine strain (Francis et al, 1953, Hennessy et al, 1955, Davenport and Hennessy, 1956, Davenport and Hennessy, 1957) It was first demonstrated by Mulder (1957, 1958) and repeatedly confirmed in other countries that persons of 70 to 80 years of age may possess antibodies to the new 1957 Asian strains This new strain will also dominate the antibody pattern of present-day children (Mulder, 1958) These antibody patterns correspond strikingly to what is known of strain prevalences (Fig 111). The same patterns have been noted in the sera of populations in the United States, England, Japan and Czechoslovakia (Davenport et al, 1955, Davenport and Hennessy, 1958, Blaskovic and Rathova, 1956) Since 1935 when studies of the age-distribution of antibodies to swine, PR8 and WS strains were first made, the age-distribution of antibodies in the population to those strains has shifted chronologically in keeping with the passage of time (*Francis et al, 1953*) These data emphasize

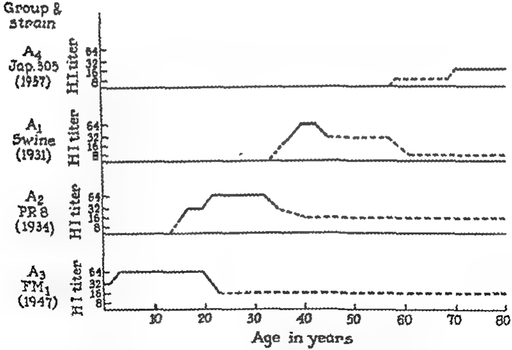

FIG 111 Age distribution of antibodies in the general population to groups of Type A *influenza virus* (Redrawn after Davenport, F M, Hennessey, A V., and Francis, T, Jr, 1953, Epidemiologic and immunologic significance of age distribution of antibody to antigenic variants of influenza virus, J Exper. Med *98*, 641-656)

the probability that a virus antigenically similar to swine influenza virus was associated with the 1918 epidemic in man, and Mulder (1957, 1958) has suggested from the time relation of antibody to Asian strains that this virus may be the agent prevalent in the pandemic of 1889-90. Moreover, it has been well demonstrated that the characteristic antibody of an age group is enhanced by vaccination with any strains of the same type, and further that a primary conditioning to a given strain can be established by vaccination with that strain as well as by infection Through these studies it has become clear that the younger segments of the population are lacking in antibody to strains which were prevalent as recently as 20 to 30 years ago The sharp strain-specific character of antibody in childhood is reflected in the uniformly high incidence at that age in succeeding epidemics caused by closely related strains; the reduced incidence above 40 or so reflects the accumulated broader, less strain-specific antibody, the immune behavior of persons in the middle range can be expected to be intermediate in character determined by the multiplicity of their experiences in relation to the antigenic character of the epidemic strain.

On the basis of this evidence variations in age incidence and epidemic recurrences can

tion Under these conditions, the hypothesis that virus variation occurs within finite boundaries also postulates that in the accession or the recession of antigens these areas of deficiency invite a back mutation, bringing into prominence again epidemic strains with major antigens characteristic of earlier strains This has now been clearly demonstrated by the presence of antibody to the 1957 Asian strains in the older segment of the present population A cycle has apparently been completed. The significance of immunologic experience accumulated earlier with antigens of apparently new strains is also reflected in the progressive decrease of incidence in persons over 30 years of age in the 1957 and in other experiences The fact that some normal children possess antibodies to the Asian 1957 strains points to the existence of that antigen in other recent strains The development of antibody to swine virus in young children infected with A-prime strains indicates that the primary swine antigen is also still prevalent New epidemics may thus represent strains with dominant antigenic characteristics differing from those of immediately preceding years but similar to major antigens of earlier strains or to secondary antigens encountered in a variety of strains Therefore, cyclic recurrences of epidemics can be expected in which the antigenic dominants of older strains, taking advantage of the increas-

is opening widely On the basis of present knowledge it seems more likely that this will constitute another rearrangement of existing antigens rather than the return of the same strain which has remained intact for an interval of many years If, however, a sustained reservoir should be demonstrated, as Mulder suggests (1958), a recurrence of the intact strain could occur The periodicity of such events should be quite irregular, governed by mutational chance and population characteristics rather than an inherent cyclic germination of the strain itself

Influenza B has been encountered less commonly in extensive, abrupt epidemics than influenza A, and there tends to be a longer interval between outbreaks Low-grade endemic and sporadic distribution seems to be rather constant The relative incidence in children is higher than in adults In one instance Magill (1945) described a high incidence of clinical illness in prisoners-of-war groups, while inapparent infection was more prominent among

genicity on a more continuous scale could play an important role in the lesser frequency of epidemics of influenza B. Cross relationships between Type-B strains appear to be more extensive, so that immunity is of greater duration Moreover, effective vaccination of human subjects against influenza B has been less affected by strain differences

Influenza C has been shown by antibody studies to be widely distributed as an infection in the population, and the recognized clinical manifestations are those of mild upper respiratory infection Up to the present only small localized prevalences and scattered cases have been specifically identified in various parts of the world

As noted elsewhere, the characteristics of influenza D have not been thoroughly mapped nor is its relationship to man and other animals—mice and swine—satisfactorily established It does not appear to belong to the

Newcastle disease group. The recovery of strains from human epidemics in Russia and serologic evidence indicate that it is prevalent as an infection. In Glasgow, Grist et al (1957) reported a new case in a 75-year-old man. In the United States in 1954 the highest frequency of antibodies was noted in the age groups of 10 to 20 years, while infants and young children had little or none (Jensen et al, 1955). In Japan the frequency of antibody varied in different districts, and this is reported also in Russia (Kuroya et al., 1953, Gorbunova, et al., 1957). Chanock et al (1958) have recently reported isolation in the United States of strains which appear to be Type II. They were recovered from children with croup. They have recovered still another virus from epidemic respiratory disease of children, which they suggest tentatively may be influenza E.

CONTROL MEASURES

Owing to the short incubation period, the abrupt onset and the high infectiousness, limitation of the spread of influenza by population control measures such as isolation and quarantine scarcely seems to be applicable. The 1918 experience in Australia, for instance, has been considered an indication that strict maritime quarantine might be effective in preventing introduction into a new community (Cumpston, 1919). In 1957 numerous incidences occurred which clearly related the introduction of influenza into unaffected countries by passengers from airplanes or by passengers or crews of infected ships. In military units the tendency to ignore interchange or transfer of men limits any possible effectiveness by such means. Nevertheless, it may be that if a severe epidemic were to be known to be approaching and a rigid quarantine were instituted, some delay could be obtained. These procedures have always encountered economic obstacles when epidemics are so commonly mild, they are also difficult to enforce administratively. It is quite clear, nevertheless, that arrangements which reduce crowding can have an influence on the rate of spread and on incidence in a given population. Here again the experience of 1957 is of interest in that the majority of the initial foci of disease appeared in groups gathered in close proximity.

Attention has been given to application of air-sterilization in such a way as to create a barrier to transmission of influenza virus. In crowded groups or in close quarters where air may be heavily contaminated by affected persons this, theoretically, should have some influence. Up to the present time, however, the use of various aerosols or ultraviolet light has not proved to be an effective means of control.

The greatest attention has been given to the development and the application of prophylactic vaccination. In this the Commission on Influenza of the Armed Forces Epidemiological Board has played a leading part. Their well-controlled investigations have continued over a period of 15 years, employing various preparations of vaccine. They have involved periods of high incidence and low incidence in such a way as to test the significance of certain vaccines in the face of outbreaks associated with strains of different degree of antigenic variation.

The approach to prevention of influenza by vaccination is based on the observations that recovery from infection is accompanied by antibody development and resistance, that in experimental animals antibody production and immunity can be induced by pararespiratory injection of active or inactive virus and that circulating antibody levels comparable with those observed in convalescence can be obtained in man by vaccination, presumably reflecting an accompanying immunity. Moreover, it has been shown that vaccination enhances the neutralizing capacity of nasal secretions in man, thus providing increased protection to the susceptible cells of the respiratory mucosa. In ferrets and mice vaccination has been shown to prevent the severe virus pneumonia produced by well-adapted strains of virus, even though susceptibility to the primary epithelial injury may remain. The effectiveness of vaccination has been clearly shown to bear a relation to amount of virus antigen administered. Recovery of swine from infection with influenza virus alone protects against combined infection with virus and bacterium, showing that the virus is the essential factor in providing immunity.

A number of early studies with active or inactive virus from various sources demonstrated the antigenic influence of vaccine, but the results in field prophylaxis were incon-

sistent owing partly, at least, to low incidence of disease, inconstant potency of vaccine and strain variation. In 1943 the now classic study with alternate placebo controls, conducted by the Commission on Influenza in ASTP units, clearly showed that subcutaneous vaccination with concentrated inactivated virus from chick allantoic fluid would prevent epidemic influenza A with an effectiveness of about 75 per cent The incidence of disease in the vaccinated was inversely related to the level of circulating antibody to the epidemic strain; this observation has been substantiated repeatedly. It was noted that the effect of vaccine became evident in 7 to 10 days after its administration when antibodies are accumulating. In some of the study units streptococcal infections were prevalent at the time influenza appeared without creating significant complications. Other reports indicated that influenza was significantly reduced in institutional populations vaccinated a year earlier

In 1945 the same vaccine which contained both Type A and Type B virus antigens was shown to be effective in prevention of epidemic influenza B At the University of Michigan and at Yale University the incidence of the disease in totally vaccinated Army and unvaccinated Navy units was compared Rates in the vaccinated were 1.1 and 0 5 per cent; in the unvaccinated 10 and 12.5 per cent, respectively, yielding an estimated effectiveness of 90 per cent or more

The foregoing results compiled by different observers were conclusive evidence that vaccination can be a practical, effective prophylactic measure The problem of strain variation was still to be reckoned with, however, and in 1947 vaccine of the same composition was found to be ineffective in an epidemic caused by the newly emergent A-prime strains The significance of strain variation in relation to immunity was thus convincingly demonstrated. This information has been extensively reviewed (Francis, 1950; 1954).

Since that time studies have continued with varied formulae of monovalent and polyvalent vaccines. The antigenic advantages of vaccines prepared with mineral oil adjuvants have been demonstrated, and limited observations of their prophylactic effect have been made (Philip et al, 1954; Boemi et al, 1955, Davenport et al, 1956). Shifts in antigenic character of Type-B strains have been evaluated; even though succeeding strains were serologically divergent, vaccination with the Lee strain of 1940 was found to give cross-protection against the later variants of 1945 and 1952. In early 1955, however, adjuvant vaccine with the Lee strain induced poor antibody response and low protection against the prevalent strain of Type B, demonstrating also significant antigenic shift in this type Table 29 summarizes results of the series of evaluations of vaccine conducted from 1943 to the present by the Commission on Influenza.

In many of these years the incidence of influenza in the study populations was low, so that excessive effort was required to separate influenza from concurrent disease of other nature It is particularly true in the studies among recruit populations in which adenovirus infections are commonly high

It should again be noted that while some reservations have been expressed [redacted] to whether influenza in vaccinated persons is adequately demonstrable by serologic procedures, a rise in titer to the homologous virus is ordinarily seen in convalescent serum with the hemagglutination-inhibition test and with the complement-fixation test as well Antibody rises have been readily observed in vaccinated persons subjected to experimental infection and in persons or animals undergoing a second experience with the same virus

The experience of 1957 was extremely informative in many ways The intensive collaborative efforts to provide vaccine with the Asian strains in the United States in the face of the oncoming epidemic was a remarkable accomplishment. Material for early initiation of vaccine studies was obtained in 2 months, and preliminary results were promptly obtained showing an effect of 40 to 75 per cent protection, depending upon the potency of the preparation (Commission on Influenza, 1957) It seems clear that older strains would not have provided high protection singly, but there are suggestions that polyvalent vaccine of earlier Type-A strains had a distinct though heterologous effect It is noteworthy that in the period of August to December

TABLE 29 SUMMARY OF VACCINE EXPERIMENTS CONDUCTED BY THE COMMISSION ON INFLUENZA

Year	Prevailing Type	Concentration of Vaccine	No Vaccinated	No Cases	Rate	No Controls	No of Cases	Rate	Protection Ratio
1943	A	5000 HU	5806	114	1.96	5776	408	7.06	3.6
1945	B	5000 HU	1150	10	0.87	2150	241	11.21	12.9
1947	A prime	3120 HU	10328	743	7.19	7615	616	8.09	1.1
1950	A prime	300 CCA	670	8	1.2	2082	78	3.7	3.1
1951	A prime	500 CCA	2596	13	0.5	5228	105	2.01	4.0
1952	B	700 CCA	207	15	7.24	430	83	19.32	2.7
1953	A prime	750 CCA	5994	57	0.95	3527	316	5.7	6.0 (corrected ratio = 8.1)
1953	A-prime	750 CCA	2616	16	0.61	4865	135	2.77	4.5
1955	B	50 CCA Adj	2000	43	2.2	2000	70	3.5	1.6 (corrected ratio = 2.2)
1957	A prime	750 CCA	1189	11	0.92	1236	62	5.1	5.5*
1957	Asian	250 CCA Mono	916	20	2.18	1448	55	3.79	1.7
1957	Asian	200 CCA Mono	775	46	5.93	806	121	15.01	2.5
1957	Asian	400 CCA Mono	649	12	1.73	1238	65	5.25	3.0
		400 CCA Poly	564	9					
1957	Asian	200 CCA Mono	1869	62	3.32	1665	126	7.61	2.3
		750 CCA Mono	1665	29	1.74				4.4
1957	Asian	200 CCA Mono	1080	43	3.98	1444	234	16.2	4.1
		750 CCA Poly without Asian strain	1031	95	9.21				1.8

HU = hemagglutinating units—pattern test
10 HU = 1 CCA approximately
* Reference to Meiklejohn, G., 1958, The effectiveness of monovalent influenza A-prime vaccine during 1957 influenza A-prime epidemic, Am J Hyg 67, 237-249

over 50 million doses of vaccine were distributed Priorities recommended under emergency conditions are important to review Among the first are the military defense forces, the medical and health personnel, those responsible for maintenance of community facilities and needs, essential industry, and persons of special susceptibility, such as those with chronic cardiac or other debilitating conditions, and pregnant mothers (Health Officers' Meeting on Asian Influenza)

The epidemic of 1947 which gave little warning and that of 1957 which was steadily traced bring into consideration two differing views of the outlook toward vaccination The one which has been a consistent perspective in the Commission studies is to seek an aggregation of the varied antigenic components representative of Type-A virus, for example, into a vaccine which will then encompass the range of variation and induce a broad antibody response to an extensive variety of strains The promise of this plan is bolstered by antigenic analysis of strains, by the age distribution of antibodies in the human population and by the fact that antibodies to newly recognized variants may be found in human serum before the variants appear epidemically (cf Epidemiology) This hypothesis also provides a hopeful basis, supported by immunologic and epidemiologic evidence, upon which to establish a well-rounded effective resistance by vaccination The group-character of convalescent antibody further points up strain interrelationships An alternate view, which seems scarcely consistent with present knowledge, is that new strains develop progressively with the appearance of

completely new dominant antigens unrelated to those of earlier times. The outlook according to this theme is that of finding the new strain and proceeding by emergency headlong procedures to provide vaccine against them in the face of the epidemic. If this is accepted, the outlook for vaccination is dark unless new methods and principles can be established. *It must be remembered that 25 years of acquaintance with influenza virus is but a short time in its history* Nevertheless, increasing evidence is developing to relate supposedly new variants, such as 1957 Asian strains, with preceding prevalences that have occurred within the life span of the present population.

Evidence has steadily accumulated to indicate that in the younger segments of the population which have had little or no experience with a given family of virus, more than one dose of vaccine at intervals of 6 weeks or more is desirable A single dose of high concentration has value for initial stimulation, but its practicality is limited by the increasing pharmacologic toxicity of increased virus content. This difficulty can be largely circumvented by giving multiple small doses at short intervals for the initial course Even with large concentrations of virus, multiple doses are needed for young inexperienced children In children or adults with some antecedent antibody to a strain, a single large dose of vaccine may produce a prompt development of high levels of homologous antibody and, simultaneously, antibody is enhanced to related strains encountered earlier (Quilligan et al , 1948, Davenport and Hennessy, 1957) Moreover, previous vaccination with related antigens may prepare the subject for increased response to vaccine containing a newly emerging strain Primary vaccination with monovalent vaccine of a new strain may condition the antibody production in such a way that antibody to it and other strains may be increased by a booster dose of a polyvalent preparation Consideration of the vaccination schedule and vaccine composition will undoubtedly add breadth and effectiveness to vaccination against influenza

Reactions to purified influenza virus vaccine are a function of virus content (Salk, *1948; Quilligan et al., 1948). In adults these* are primarily the localized foreign protein reaction with soreness, redness, swelling of mild character and limited duration similar to those observed *with typhoid vaccine. In children*, constitutional effects with considerable fever of 24 hours' duration may be seen with a full-scale dose. A certain proportion of adults (3 to 4%) may react similarly When increased numbers of severe local and constitutional *reactions are observed in adults*, other causes than vaccine per se should be considered, pyrogenic bacterial bodies contaminating the vaccine, excessive content of virus or extraneous protein. The vaccine ordinarily should not contain egg albumin; allantoic fluid itself is of low antigenic power; chicken tissue is not present in the material Nevertheless, severe anaphylacticlike reactions have been observed in persons with known natural sensitization to feathers, eggs, etc. They should not be injected with a full dose of vaccine from egg fluid It should be given in graded doses or omitted Repeated inoculations of vaccine have not been observed to cause sensitization White mineral oil adjuvant vaccines given intramuscularly elicit little local reaction and no systemic reaction even in children given an adult dose Preparations emulsified with Arlacel A in Drakeol 6 made for the Commission on Influenza have been used in large numbers of persons To date, a cyst has practically never been encountered, except with one experimental preparation which contained an impure emulsifying agent. No neurologic difficulties occur with such preparations There is evidence that subcutaneous inoculation may be more irritating locally. These vaccines offer much promise, but they have had only limited evaluation. However, serologic evidence clearly demonstrates that smaller amounts of virus in adjuvant produce more extended and higher antibody levels than aqueous vaccine preparation

From the first experiments with pararespiratory vaccines *indications have been* that reasonable antibody responses can be obtained with intracutaneous as well as subcutaneous or intramuscular inoculation (Francis and Magill, 1937) Much of present procedure has been based on military preference *Intracutaneous injections may* be multiple and repetitive It should be possible to obtain

adequate immunization by this route with attention to proper combinations of initial dose and intervals. Results are generally consistent in showing that a single 0.1 cc. intracutaneously is a less effective stimulus than 1.0 cc. subcutaneously. In children this defect is even more important because of their lack of previous conditioning experience. There are no reports of adequate field trials of protective effect of intracutaneous vaccine but they may be forthcoming from 1957 studies.

On the basis of experimental evidence vaccination should be of even greater effectiveness in preventing viral pneumonia injury than in preventing mild respiratory illness. Because of the comparative mildness of usual epidemics, there is little information regarding this feature. Pneumonia among vaccinated members of study populations has been very uncommon, and the deaths reported from the 1957 experience in the United States have been essentially limited to unvaccinated persons.

The duration of effective resistance resulting from single vaccination is not known nor is it adequately known after natural infection. Undoubtedly, the composition of vaccine and its potency are important factors. Mention has been made of protective effect apparently persisting for a year to significant degree. After a single moderate dose, antibody levels tend to decline by about one third in 3 or 4 months and to about one half the peak level in a year, at that time titers remain well above those in the rest of the general population (Salk et al., 1945). In children titers decline to very low levels more rapidly, but this depends upon the levels achieved after vaccination, that the conditioning effect remains can be demonstrated by revaccination. In one study cases among vaccinated children were seen to develop in those whose antibody had fallen extensively in the 2 months between vaccination and exposure. In certain experimental studies it was observed that 32 per cent of persons vaccinated 4½ months earlier exhibited clinical illness when subjected to Type A virus by inhalation, while 16 per cent of those vaccinated 2 weeks prior to testing had signs of illness but none with fever greater than 100° F. Fifty per cent of controls had characteristic illness. Vaccination against Type B apparently provided firmer resistance, 41 per cent of controls had illness, but only 10 per cent of vaccinated, and none with fever above 100° F., those vaccinated 4½ months earlier were as resistant as the groups vaccinated either 4 weeks before challenge or both times. It seems probable that increased use of multiple doses will have a valuable effect in lengthening durable immunity (cf. reviews, Francis, 1950, 1954).

The approach to immunization by modified respiratory infection with live attenuated virus has had steady consideration. It has certain theoretical advantages in that the natural route of infection is employed, the antigenic dose is increased by virus multiplication, and what influence the cellular reaction may provide is presumably obtained. In this country, studies with experimental infection suggested that as good or better protection was obtained after subcutaneous vaccination than after intranasal spray eliciting clinical illness. Live virus vaccination has been studied most extensively by Soviet investigators. The epidemiologic experiments conducted from 1938 to 1954 in different towns indicated the lowering by 1.5 to 3 times the incidence of influenza among persons vaccinated with live vaccine (Smorodintsev and Zhdanov, 1957). A study was begun in December 1953, at the onset of an epidemic of influenza A-prime involving about 15,000 workers in a cotton factory (Unanov, 1957). A live polyvalent vaccine containing strains of Types A, A-prime and B was used. A clinical incidence of 8.8 per cent was observed in the vaccinated and 44.2 per cent in the unvaccinated during the sharp epidemic period. In November, 1954, a study in 16,000 of this same population was undertaken with polyvalent vaccine of the same composition. An epidemic of influenza B began early in December and reached a sharp maximum in the latter half of January. Two per cent developed mild illness of short duration with fever, headache and respiratory catarrh from the administration of vaccine. Four groups were observed with clinical recording of illness during the epidemic prevalence which, according to serologic tests, was not more than 50 per cent influenza. The results reported are as follows

Groups	No	Cases of Influenza and Catarrh		Complications No
		No	Per Cent	
Vaccinated in 1954	6056	583	9.6	6
Vaccinated in 1953 & 1954	3931	476	12 2	5
	9987	1059	10 6	11
Vaccinated in 1953	1901	537	28.2	32
Unvaccinated	4972	1769	35.4	4
	6873	2306	33 6	36

Two vaccinations at a year's interval had no added effect, while vaccination in the preceding year had no demonstrable influence The maximum effect of 3 8-fold reduction occurred during the major epidemic period Serologic studies indicated 72 per cent of tested cases were influenza B; antibody rises to Type A were noted in 7 8 per cent and to A-prime in 10 5 per cent Data of this nature are not consistent, because effects from 1.5 to 5 times are reported from different studies Smorodintsev and Zhdanov (1957) reviewing the problem state that poor effects were obtained with the vaccine in 1955 because the epidemic Type B strains differed, as in the United States experience, poor results were also obtained when A-prime strains appeared in 1949. Observations of this nature would not be in keeping with the concept of interference protection. These authors present an excellent review of the problems involved in production of a satisfactory live virus vaccine on a large scale; they comment especially on the unsatisfactory results related to irregularities in desiccation, in infectious potency of strains, and in lack of quality control Strains for use are best adapted by passage in human susceptibles or in cultures of human embryonic lung They are still too virulent for children below 7 or 8 years Apparently, multiple strains must be considered to cover the antigenic range. Emphasis is given to the need for extensive inhalation to obtain consistent antibody production. The effect is apparently immunologic rather than interference, since infection with antigenically deviant strains is not prevented, and the effect begins in 7 to 10 days when antibodies appear. Its duration is obviously short. In fact, the selection of strains and other problems appear to create problems somewhat more complex than for inactive vaccine. In the autumn of 1957, 10 million doses of fourth egg passage virus of little attenuation were prepared. In this country much more inactivated virus vaccine was produced

Other studies have emphasized the disadvantages of too much attenuation Under these conditions, antibody response in adults is very uncertain In children, however, old strains may be effective without significant injury, although the Russian observers state that even these may cause marked clinical disturbance in young children.

One possibility, inadequately explored, is the intranasal spray of purified inactive virus Antibody response may be obtained, but a greater effect is obtained with smaller amounts of inactive virus subcutaneously (Quilligan and Francis, 1947).

Prophylaxis by means of intranasal spray of immune serum has been recommended by Smorodintseff, especially In one study he and associates reported a reduction in incidence of influenza under epidemic conditions from 82 per 1,000 in controls to 8 per 1,000 in the treated group by spraying with 2 0 cc of immune horse serum (Smorodintsev et al, 1940). This of itself constitutes a risk in sensitization. It has also been employed in treatment by the Russian investigators In extensive studies conducted by the Commission on Influenza with large individual inhalations of sprayed human convalescent serum and induced infection, no evidence of protection or therapeutic effect was observed

At present no effective prophylactic drug has been demonstrated, although there are numerous suggestions of preventive chemicals under experimental conditions in eggs, cultures, or mice. The use of bacterial vaccines as possible preventives of complications has not been seriously explored

REFERENCES

Abbott, S W, 1890, Influenza epidemic of 1889-90 Board of Health, Mass, Report, *21*, 307-383

Ackermann, W W, 1951, Concerning the relation of the Krebs cycle to virus propagation, J Biol Chem *189*, 421-428

Ackermann, W W, and Maassab, H F, 1954a, Growth characteristics of influenza virus the influence of a sulfonic acid, J Exper Med *99*, 105-117

———, 1954b, Alcune caratteristiche dello sviluppo del virus della polmonite nel neonato (Sendai), Minerva med *45*, 1518-1521

———, 1954c, Growth characteristics of influenza virus Biochemical differentiation of stages of development, J Exper Med *100*, 329-339

Ada, G L, and Perry, B T, 1956, Influenza virus nucleic acid relationship between biological characteristics of the virus particle and properties of the nucleic acid, J Gen Microbiol *14*, 623-633

Andrewes, C H, 1950, Adventures among viruses II Epidemic Influenza New England J Med *242*, 197-203

———, 1957, Factors in virus evolution in Advances in Virus Research, vol *4*, pp 1-24, New York, Acad Press

Andrewes, C H, Laidlaw, P P, and Smith, W, 1934, The susceptibility of mice to the viruses of human and swine influenza, Lancet *2*, 859-862

Bang, F B, 1958, Personal communication

Baron, S, and Jensen, K E, 1955, Evidence for genetic interaction between non-infectious and infectious influenza A viruses, J Exper Med *102*, 677-697

Bernkopf, H, 1950, Study of infectivity and hemagglutination of influenza virus in deembryonated eggs, J Immunol *65*, 571-583

Beveridge, W I B, and Burnet, F M, 1946, The cultivation of viruses and rickettsiae in the chick embryo Medical Research Council, Special report series No 256 London, His Majesty's Stat Off 92 pp

Blaškovič, D, and Rathová, V, 1956, Hladiny protilatok proti virusom chripky A, A', B, C, virusu chripky prasiec (shopeiowa 15) u obyvatel'stva Československa, Česk Epidem, Mikro, Immunol *5*, 115-124

Boand, A V, Jr, Kempf, J E, and Hanson, R J, 1957, Phagocytosis of influenza virus I In vitro observations J Immunol *79*, 416-421

Boemi, G, Balducci, D, and Mongia, L, 1955, Ricerche sierologiche e incidenza dei virus dell' influenza A, B, C, e virus APC in soggetti trattati con vaccino antinfluenzale emulsionato, Riv Ist, sieroterap ital *30*, 316-343

Brightman, I J, 1936, Recovery of a filtrable virus from children with influenza II The experimental disease in ferrets Am J Dis Child *52*, 78-95

Burnet, F M, 1940, Influenza virus infections of the chick embryo by the amniotic route I General character of the infections Australian J Exper Biol & M Sc *18*, 353-360

———, 1953, Recent work on the intrinsic qualities of influenza virus Somatic and genetic aspects Bull World Health Organ *8*, 661-682

Burnet, F M, and Bull, D R, 1943, Changes in influenza virus associated with adaptation to passage in chick embryos, Australian J Exper Biol & M Sc *21*, 55-69

Burnet, F M, and Lind, P E, 1951, A genetic approach to variation in influenza viruses 3 Recombination of characters in influenza virus strains used in mixed infections J Gen Microbiology *5*, 59-66

Burnet, F M, McCrea, J F, and Stone, J D, 1946, Modification of human red cells by virus action. I The receptor gradient for virus action in human red cells Brit J Exper Path *27*, 228-236

Cairns, H J F, Farekas de St Groth, and Edney, M, 1952, Quantitative aspects of influenza virus multiplication I Production of "incomplete virus" J Immunol *69*, 155-181

Callender, G R, 1929, Pathology of the acute respiratory diseases Med Dept of U S Army in the World War, vol 12, Washington, U S Gov't Print Off

Chanock, R M, et al, 1958, Newly recognized myxoviruses from children with respiratory disease, New England J Med *258*, 207-213

Chu, C M, Andrewes, C H, and Gledhill, A W, 1950, Influenza in 1948-1949, Bull World Health Organ *3*, 187-214

Collins, S D, 1944, Age and sex incidence of influenza in the epidemic of 1943-44 with comparative data for preceding outbreaks, Pub Health Rep *59*, 1483-1503

Collins, S D, and Lehmann, J L, 1957, Influenza epidemics during 1951-56 with a review of trends, Pub Health Rep *72*, 771-780

Commission on Influenza, 1943, Unpublished studies of infection in human volunteers

Commission on Influenza, Armed Forces Epidemiological Board, 1957, Vaccination against Asian influenza, basis for recommendations and a preliminary report on efficacy, J A M A *165*, 2055-2058

Communicable Disease Center Influenza Reports, 1957-58, U S Pub Health Service, Bureau of State Services, C D C, Atlanta, Georgia

Communicable Disease Center Influenza Reports and Maps, 1957, Public Distribution, Nov 1957, Chart I and II

Cumpston, J H L, 1919, Influenza and maritime quarantine in Australia Quarantine Service Publication No 18, Commonwealth of Australia

Davenport, F M, and Francis, T, Jr, 1951, A comparison of the growth curve of adapted and

unadapted lines of influenza virus, J Exper Med *93*, 129-137
Davenport, F M, and Hennessy, A V, 1956, A serologic recapitulation of past experiences with influenza A, antibody response to monovalent vaccine, J Exper Med *104*, 85-97
———, 1957, *Predetermination by infection* and by vaccination of antibody response to influenza virus vaccines, J Exper Med *106*, 835-850
———, 1958, Unpublished observations
Davenport, F M, Hennessy, A V, and Francis, T, Jr, 1953, Epidemiologic and immunologic significance of age distribution of antibody to antigenic variants of influenza virus, J Exper Med *98*, 641-656
Davenport, F M, Hennessy, A V, Houser, H B, and Cryns, W F, 1956, An evaluation of an adjuvant influenza virus vaccine tested against influenza B in 1954-55, Am J Hyg *64*, 304-314
Davenport, F M, Hennessy, A V, Stuart-Harris, C H, and Francis, T, Jr, 1955, Epidemiology of influenza Comparative serological observations in England and the United States Lancet *2*, 469-473
Dawson, I M, and Elford, W J, 1949, Electron microscope studies on interaction of certain viruses *with fowl red cell membranes, Nature, London*, *163*, 63-64
de Torregrosa, M V, and Francis, T, Jr, 1941, The intracerebral infection of mice with hemophilus influenzae as an index of strain virulence and the protective value of *immune serum, J Infect Dis* *68*, 59-66
Elford, W J, Andrewes, C H, and Tang, F F, 1936, The sizes of the viruses of human and swine influenza as determined by ultra filtration, Brit J Exper Path *17*, 51-53
Finter, N B, Liu, O C, and Henle, W, 1955, Studies on host-virus interactions in the chick embryo—influenza virus system X An experimental analysis of the Von Magnus phenomenon J Exper Med *101*, 461-478
Francis, T, Jr, 1934, Transmission of influenza by a filterable virus, Science *80*, 457-459
———, 1937, Epidemiological studies in influenza, J Am Pub Health A *27*, 211-225
———, 1940a, A new type of virus from epidemic influenza, Science *92*, 405-408
———, 1940b, Intranasal inoculation of human individuals with the virus of epidemic influenza, Proc Soc Exper Biol & Med *43*, 337-339
———, 1942, Factors conditioning resistance to epidemic influenza *in* Harvey Lectures, Series 37, (1941-1942) pp 69-99, New York, The Harvey Society
———, 1943, *Epidemiology* of influenza, J.A.M.A *122*, 4-8
———, 1945, The development of the 1943 vaccination study of the Commission on Influenza, Am J Hyg *42*, 1
———, 1947, *Dissociation of hemagglutinating and* antibody measuring capacities of influenza virus, J Exper Med *85*, 1-7
———, 1950, Immunity and vaccination in influenza *in* Doerr, R, and Hollauer, C (eds), Handbuch der Virus forschung, Vienna, Springer, *2 (Suppl)*, *66-86*
———, 1952, Significance of antigenic variation of influenza viruses in relation to vaccination in man, Fed Proc *2*, 808-812
———, 1953, Influenza the newe acquayantance, Ann Int Med *39*, *203-221*
———, 1953, Vaccination against influenza, Bull World Health Organ *8*, 725-741
———, 1955, The current status of the control of influenza, Ann Int Med *43*, 534-538
Francis, T, Jr, Davenport, F. M, and Hennessy, A V, 1953, A serological recapitulation of human infection with different strains of influenza virus, Tr A Am Physicians, *66*, 231-239
Francis, T, Jr, and de Torregrosa, M. V, 1945, Combined infection of mice with H influenza and influenza virus by the intranasal route, J Infect Dis *76*, 70-77
Francis, T, Jr, and Magill, T P, 1935, Cultivation *of human influenza virus* in an artificial medium, Science *82*, 353-354
———, 1937, The antibody response of human subjects vaccinated with the virus of human influenza, J Exper Med *65*, 251-259
— [illegible]
Fr [illegible]
Fr [illegible]
Brown, P N, 1944, Immunity in human subjects artificially infected with influenza virus Type B, Am J Pub Health *34*, 317-334
Francis, T, Jr, Pearson, H E, Sullivan, E R, and Brown, P N, 1943, The effect of subcutaneous vaccination with influenza virus upon the virus reactivating capacity of nasal secretions, Am J Hyg *37*, 294-300
Francis, T, Jr, Quilligan, J J, Jr, and *Minuse, E*, 1950, Identification of another epidemic respiratory disease, Science *112*, 495-497
Francis, T, Jr, Salk, J E, and Brace, W. M, 1946, The protective effect of vaccination against epidemic influenza B, J.A.M.A *131*, 275-278,
Francis, T, Jr, and Stuart-Harris, C H, 1938, Studies on the nasal histology of epidemic influenza virus infection in the ferret I. The development and repair of the nasal lesion J Exper Med *68*, 789-809
Friedwald, W F, 1943, The immunological response to influenza virus infection as measured by the complement-fixation test Relation of the complement-fixing antigen to the virus particle J Exper Med *78*, 347-366
Friedwald, W. F, Pickels, E G, 1944, Centrifugation and ultrafiltration studies on allantoic fluid preparations of influenza virus, J Exper Med *79*, *301-317*
Frisch-Niggemeyer, W, and Hoyle, L, 1956, The nucleic acid and carbohydrate content of influenza virus A and of virus fractions produced by ether disintegration, J. Hyg *54*, 201-212

Fukumi, H, Sunakawa, S, Takaki, A., and Tanaka, K., 1951, Influenza C in Japan, Jap M J *4*, 139-141

Gerber, P, Hamre, D, and Loosli, C G, 1956, Antigenic variants of influenza A virus (PR8 strain) II Serological and immunological characteristics of variants derived from variants J Exper Med *103*, 413-424

Gerngross, O A., 1957, Peculiarities of the 1956 influenza outbreak in Vladivostok due to B virus, Problems of Virology, *2*, 71-75

Ginsberg, H S., and Horsfall, F L, Jr, 1949 A labile component of normal serum which combines with various viruses Neutralization of infectivity and inhibition of hemagglutination by the component J Exper Med *90*, 475-495

Goodpasture, E W., 1919, The significance of certain pulmonary lesions in relation to the etiology of influenza, Am J M Sc *158*, 863-870

Goodpasture, E W, and Burnett, F L, 1919, Pathology of pneumonia accompanying influenza, U S Nav M Bull *13*, 177

Gorbunova, A G, Gerngross, V M, Anorisova, V M., and Bukrinskaia, A G, 1957, Strains of influenza virus of Type D isolated in Vladivostok and their role in the etiology of the 1956 outbreak, Problems of Virology, *2*, 76-84

Gotlieb, T, and Hirst, G K, 1954, Experimental production of combination forms of virus, formation of doubly antigenic particles from influenza A and B viruses and study of ability of individual particles of X virus to yield two separate strains, J Exper Med *99*, 307-320

Grist, N R, Sommerville, R G, and Carson, H G., 1957, Respiratory virus infections in hospital and in general practice 1954-6, Brit M J *1*, 378-380

Hall, M W, 1928, Inflammatory diseases of the respiratory tract (bronchitis, influenza, bronchopneumonia, lobar pneumonia) Med Dept of the U S Army in the World War, vol 9, Communicable and other diseases

Hanig, M, 1948, Electrokinetic change in human erythrocytes during adsorption and elution of PR8 influenza virus, Proc Soc Exper Biol & Med *68*, 385-392

Hanson, R J, Kempf, J E, and Boand, A V, Jr, 1957, Phagocytosis of influenza virus II Its occurrence in normal and immune mice J Immunol *79*, 422-427

Hare, R, Hamilton, J, and Feasby, W R, 1943, Influenza and similar respiratory infections in a military camp over a period of three years, Canad J Pub Health *34*, 453-464

Harford, C G., Smith, M R, and Wood, W B, Jr, 1946, Sulfonamide chemotherapy of combined infection with influenza virus and bacteria, J Exper Med *83*, 505-518

Hartman, R E, and Ackermann, W W, 1954, Newborn pneumonitis virus (Sendai) II Physical properties Fed Proc *13*, 496

Health Officers' Meeting on Asian Influenza, 1957, Public Health Reports, *72*, 998-1000

Henle, G, Girardi, A, and Henle, W, 1955, A nontransmissible cytopathogenic effect of influenza virus in tissue culture accompanied by formation of non-infectious hemagglutinins, J Exper Med *101*, 25-41

Henle, G., and Henle, W., 1946a, Studies on the toxicity of influenza viruses I The effect of intracerebral injection of influenza viruses J. Exper Med *84*, 623-637

Henle, W, 1953, Multiplication of influenza virus in the entodermal cells of the allantois of the chick embryo *in* Advances in Virus Research, vol 1, pp 141-227, New York, Acad Press

Henle, W, and Henle, G., 1946b, Studies on the toxicity of influenza viruses II The effect of intra-abdominal and intravenous injection of influenza viruses J Exper Med *84*, 639-660

Henle, W, Henle, G, Stokes, J, Jr, and Mario, E K, 1946, Experimental exposure of human subjects to viruses of influenza, J Immunol *52*, 145-165

Hennessy, A V, Davenport, F M, and Francis, T, Jr, 1955, Studies of antibodies to strains of influenza virus in persons of different ages in sera collected in a postepidemic period, J Immunol *75*, 401-409

Henry, C, and Youngner, H C, 1957, Influence of normal animal sera on influenza viruses in cultures of trypsin-dispersed monkey kidney cells, J Immunol *78*, 273-281

Hers, J F P., 1955, The Histopathology of the Respiratory Tract in Human Influenza, Leiden, H E Stenfert Kroese N V.

Hers, J F P, and Mulder, J, 1951, Rapid tentative post-mortem diagnosis of influenza with the aid of cytological smears of the tracheal epithelium, J Path & Bact *63*, 329-332

———, 1953, Mucosal epithelium of respiratory tract in muco-purulent bronchitis caused by Haemophilus influenzae, J Path & Bact *66*, 103-108

Hirsch, A, 1883-1886, Handbook of Geographical and Historical Pathology, 3 vols Trans by Chas Creighton, London, The New Sydenham Soc

Hirst, G K, 1941, The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus, Science *94*, 22-23

———, 1942a, Adsorption of influenza hemagglutinins and virus by red blood cells, J Exper Med *76*, 195 209

———, 1942b, The quantitative determination of influenza virus and antibodies by means of red cells agglutination, J Exper Med *75*, 49-64

———, 1943, Adsorption of influenza virus on cells of the respiratory tract, J Exper Med *78*, 99-109

———, 1945, Direct isolation of human influenza virus in chick embryos, Proc Soc Exper Biol & Med *58*, 155-157

———, 1950, The relationship of the receptors of a new strain of virus to those of the mumps-NDV-influenza group, J Exper Med *91*, 177-184

———, 1954, Studies on double infections with influenza virus, *in* Hartman, F W, Horsfall, F L., Jr., and Kidd, J G, (eds), The Dynamics of Virus and Rickettsial Infections, pp 45-49, New York, Blakiston

Horsfall, F L, Jr, 1954, On the reproduction of influenza virus quantitative studies with procedures

unadapted lines of influenza virus, J Exper Med *93*, 129-137
Davenport, F M, and Hennessy, A V, 1956, A serologic recapitulation of past experiences with influenza A, antibody response to monovalent vaccine, J Exper Med *104*, 85-97
———, 1957, Predetermination by infection and by vaccination of antibody response to influenza virus vaccines, J Exper Med *106*, 835-850
———, 1958, Unpublished observations
Davenport, F M, Hennessy, A V, and Francis, T, Jr, 1953, Epidemiologic and immunologic significance of age distribution of antibody to antigenic variants of influenza virus, J Exper Med *98*, 641-656
Davenport, F M, Hennessy, A V, Houser, H B, and Cryns, W F, 1956, An evaluation of an adjuvant influenza virus vaccine tested against influenza B in 1954-55, Am J Hyg *64*, 304-314
Davenport, F M, Hennessy, A V, Stuart-Harris, C H, and Francis, T, Jr, 1955, Epidemiology of influenza Comparative serological observations in England and the United States Lancet *2*, 469-473
Dawson, I M, and Elford, W J, 1949, Electron microscope studies on interaction of certain viruses with fowl red cell membranes, Nature, London, *163*, 63-64
de Torregrosa, M V, and Francis, T, Jr, 1941, The intracerebral infection of mice with hemophilus influenzae as an index of strain virulence and the protective value of immune serum, J Infect Dis *68*, 59-66
Elford, W J, Andrewes, C H, and Tang, F F, 1936, The sizes of the viruses of human and swine influenza as determined by ultra filtration, Brit *J Exper Path 17, 51-53*
Finter, N B, Liu, O C, and Henle, W, 1955, Studies on host-virus interactions in the chick embryo—influenza virus system X An experimental analysis of the Von Magnus phenomenon J Exper Med *101*, 461-478
Francis, T, Jr, 1934, Transmission of influenza by a filterable virus, Science *80, 457-459*
———, 1937, Epidemiological studies in influenza, J Am Pub Health A *27*, 211-225
———, 1940a, A new type of virus from epidemic *influenza, Science 92, 405-408*
———, 1940b, Intranasal inoculation of human individuals with the virus of epidemic influenza, Proc Soc Exper Biol & Med *43*, 337-339
———, 1942, Factors conditioning resistance to epidemic influenza *in* Harvey Lectures, Series 37, (1941-1942) pp 69-99, New York, The Harvey Society
———, 1943, Epidemiology of influenza, J.A.M.A *122*, 4-8
———, 1945, The development of the 1943 vaccination study of the Commission on Influenza, Am J Hyg *42*, 1
———, 1947, Dissociation of hemagglutinating and antibody measuring capacities of influenza virus, J Exper Med *85*, 1-7
———, *1950, Immunity* and vaccination in influenza *in* Doerr, R, and Hollauer, C (eds), Handbuch der Virus forschung, Vienna, Springer, *2* (Suppl), 66-86
———, *1952*, Significance of antigenic variation of influenza viruses in relation to vaccination in man, Fed Proc *2*, 808-812
———, 1953, Influenza the newe acquayantance, Ann Int. Med *39*, 203-221.
———, 1953, Vaccination against influenza, Bull *World Health Organ 8, 725-741.*
———, 1955, The current status of the control of influenza, Ann Int Med *43*, 534-538
Francis, T, Jr, Davenport, F M, and Hennessy, A V, *1953, A serological recapitulation of human* infection with different strains of influenza virus, Tr A Am Physicians, *66*, 231-239
Francis, T, Jr, and de Torregrosa, M V, *1945*, Combined infection of mice with H influenza and influenza virus by the intranasal route, J Infect Dis *76, 70-77*
Francis, T, Jr, and Magill, T P, 1935, Cultivation of human influenza virus in an artificial medium, Science *82, 353-354*
———, 1937, The antibody response of human subjects vaccinated with the virus of human influenza, J Exper Med *65, 251-259.*
———, 1938, Antigenic differences in strains of epidemic influenza virus II Cross-immunization on tests in mice Brit J Exper Path *19*, 284-293
Francis, T, Jr, and Moore, A E, 1940, A study of the neurotropic tendency in strains of the virus of epidemic influenza, J. Exper Med *72*, 717-728
Francis, T, Jr, Pearson, H E, Salk, J E, and Brown, P N, 1944, Immunity in human subjects artificially infected with influenza virus Type B, Am J Pub Health *34*, 317-334
Francis, T, Jr, Pearson, H E, Sullivan, E R, and Brown, P N, 1943, The effect of subcutaneous vaccination with influenza virus upon the virus reactivating capacity of nasal secretions, Am J Hyg *37, 294-300*
Francis, T, Jr, Quilligan, J J, Jr, and Minuse, E, 1950, Identification of another epidemic respiratory disease, Science *112, 495-497*
Francis, T, Jr, Salk, J E, and Brace, W M, 1946, The protective effect of vaccination against epidemic influenza B, J.A.M.A *131, 275-278*
Francis, T, Jr, and Stuart-Harris, C H, 1938, Studies on the nasal histology of epidemic influenza virus infection in the ferret I The development and repair of the nasal lesion J Exper Med *68*, 789-809
Friedwald, W F, 1943, The immunological response to influenza virus infection as measured by the complement-fixation test Relation of the complement-fixing antigen to the virus particle J Exper Med *78*, 347-366
Friedwald, W. F, Pickels, E G, 1944, Centrifugation and ultrafiltration studies on allantoic fluid preparations of influenza virus, J Exper Med *79*, 301-317
Frisch-Niggemeyer, W, and Hoyle, L, 1956, The nucleic acid and carbohydrate content of influenza virus A and of virus fractions produced by ether disintegration, J. Hyg *54*, 201-212

Mulder, J., and Verdonk, G J, 1949, Studies on the pathogenesis of a case of influenza-A pneumonia of three days' duration, J Path & Bact *61*, 55-61

Murphy, J S, and Bang, F B, 1952, Observations with the electron microscope on cells of the chick chorio-allantoic membrane infected with influenza virus, J Exper Med. *95*, 259-268.

Nishikawa, F., and Fukumi, H, 1954, Shape and size of hemagglutinating virus of mice (HUM), Jap J M Sc & Biol *7*, 513-522

Opie, [redacted] L., Blake, F G., Small, J C., and Rivers, T M., 1921, Epidemic Respiratory Disease The Pneumonias and Other Infections of the Respiratory Tract, Accompanying Influenza and Measles St Louis, Mosby 402 pp

Parker, F., Jr., Jolliffe, L S., Barnes, M W., and Finland, M, 1946, Pathologic findings in lungs of 5 cases from which influenza virus was isolated, Am. J Path *22*, 797-819

Pearson, H E., 1944, Distribution of influenza virus Type A in infected eggs and the survival of virus under certain conditions of storage, J Bact *48*, 369-378

Pearson, H E, and Enders, J F, 1941, Cultivation of influenza A virus in roller tubes, Proc Soc Exper Biol & Med *48*, 140-143

Pfeiffer, R, 1892, Vorläufige Mitteilungen über die Erreger der Influenza, Deutsche med Wchnschr *18*, 28.

Philip, R N., Bell, J A., Davis, D J, Beem, M O, and Beigelman, [redacted] M, 1954, Epidemiological studies on influenza [redacted] familial and general population groups I Preliminary report on studies with adjuvant vaccine Am J Pub Health *44*, 34-42

Quilligan, J J, Jr, and Francis, T, Jr, 1947, Serological response to intranasal administration of inactive influenza virus in children, J Clin Invest *26*, 1079-1087

Quilligan, J J, Jr, Minuse, E, and Francis, T, Jr, 1948, Homologous and heterologous antibody response of infants and children to multiple injections of a single strain of influenza virus, J Clin Invest *27*, 572-579

———, 1954, Type C influenza virus, II Intranasal inoculation of human individuals J Lab & Clin Med *43*, 43-47

Rickard, E R, and Francis, T, Jr, 1938, Demonstration of lesions and virus in lungs of mice receiving large intraperitoneal inoculations of epidemic influenza virus, J Exper Med *67*, 953-972

Salk, J E, 1948, Reactions to concentrated influenza virus vaccine, J Immunol *58*, 369-395

———, 1952, An interpretation of the significance of influenza virus variation for the development of an effective vaccine, Bull New York Acad Med *28*, 748-765

Salk, J E, Pearson, H E, Brown, P N, Smyth, C J, and Francis, T, Jr, 1945, Immunization against influenza with observations during an epidemic of influenza A one year after vaccination, Am J Hyg *42*, 307-322

Sano, T, Nutsu, I, and Nakagawa, I, 1953, Newborn virus pneumonitis (Type Sendai) I The clinical observation Tohoku J Exper Med *58*, 56

Sasahara, J, 1955, Studies on the HVJ (Hemagglutinating virus of Japan) newly isolated from the swine, Nat Inst of Animal Health, Tokyo, Report No 30, pp 34-38

Scadding, J G, 1937, Lung changes in influenza, Quart J Med *6*, 425-465

Schäfer, W, 1955, Vergleichende sero-immunologische Untersuchungen über die Viren der Influenza and klassischen Geflügelpest Ztschr Naturforsch *10B*, 81-91

Sharp, D G, Taylor, A R, McLean, I W., Beard, D, and Beard, J W, 1944, Sedimentation velocity and electron micrographic studies of influenza viruses A (PR8 strain) and [redacted] (Lee strain) and the swine influenza virus, J Biol Chem *156*, 585-600

Shope, R E, 1931, Swine influenza III Filtration experiments and etiology J Exper Med *54*, 373-385

———, 1934, The infection of ferrets with swine influenza virus, J Exper Med *60*, 49-61

———, 1936, The influenzas of swine and man Harvey Lectures, Series 31, pp 183-213

———, 1938, Serological evidence for the occurrence of infection with human influenza virus in swine, J Exper Med *67*, 739-748

———, 1941a and b, The swine lungworm as a reservoir and intermediate host for swine influenza virus I The presence of swine influenza in healthy and susceptible pigs II The transmission of swine influenza virus by the swine lungworm J Exper Med *74*, 41-47, 49-68

———, 1943a and b, The swine lungworm as a reservoir and intermediate host for swine influenza virus III Factors influencing transmission of the virus and the provocation of influenza IV The demonstration of masked swine influenza virus in lungworm larvae and swine under natural conditions J Exper Med *77*, 111-126, 127-138

———, 1954, Ecology and virus reservoirs *in* Hartman, F W., Horsfall, F L, Jr, and Kidd, J G, (eds), The Dynamics of Virus and Rickettsial Infections, pp 125-141, New York, Blakiston

Smith, W, and Andrewes, C H, 1938, Serological races of influenza virus, Brit J Exper Path *19*, 293-314

Smith, W, Andrewes, C H, and Laidlaw, [redacted] P, 1933, A virus obtained from influenza patients, Lancet *2*, 66-68

Smorodintsev, A A, Gulamow, A G, and Tschalkina, O M, 1940, Über die spezifische Prophylaxe der epidemischen Grippe durch Inhalation antigrippischen Serums, Ztschr Klin Med *138*, 756-765

Smorodintsev, A A, and Zhdanov, V M, 1957,

Sc

American Medical Association, Council on Drugs, 1957, Antibiotics and influenza, J A M A *165*, 58-59

Stone, J D, 1948, Prevention of virus infection with enzyme of *V cholerae* I Studies with viruses of

which enumerate infective and hemagglutinating virus particles, J Exper Med *100*, 135-161

Horsfall, F L, Jr, Lennette, E H, Rickard, E R, Andrewes, C H, Smith, W, and Stuart-Harris, C H, 1940b, The nomenclature of influenza, Lancet *2*, 413-414

Hoyle, L, and Fairbrother, R W, 1937, Antigenic *structure of influenza viruses, the preparation of* elementary body suspensions and the nature of the complement-fixing antigen, J Hyg *37*, 512-520

Hoyle, L, and Frisch-Niggemeyer, W., 1955, The disintegration of influenza virus particles on entry *into the host cell Studies with virus labelled with* radiophosphorus, J Hyg *53*, 474-486

Hubble, D, and Osborn, G R, 1941, Acute bronchiolitis in children, Brit M J *1*, 107-110

Isaacs, A, and Andrewes, C H, 1951, The spread of *influenza evidence from 1950-1951, Brit M J 2*, 921-927

Isaacs, A, Depoux, R, and Fiset, P, 1954, The viruses of the 1952-3 influenza epidemic, Bull World Health Organ *11*, 967-979

Ishida, N, 1957 [Discussion new respiratory and ocular viruses], Ann New York Acad Sc *67*, 299-300

Ivánovics, G, Horváth, S, and Szollosy, E, 1954, The influenza virus adsorbing capacity of the vas*cular endothelium of various mammals Acta Mi*crobiologica Acad Scient Hung *2*, 121-129

Jensen, K E, 1956, Influenza *in* Diagnostic Procedures for Virus and Rickettsial Disease, pp 241-262, New York, Am Public Health Assoc

———, *1957, The nature of serological relationships among influenza viruses in* Advances in Virus Research, vol 4, pp 279-310, New York, Acad Press

Jensen, K E, Davenport, F M, Hennessy, A V, and Francis, T, Jr, 1956, Characterization of influenza antibodies by *serum absorption*, J Exper Med *104*, 199-209

Jensen, K E, and Francis, T, Jr, 1953, The antigenic composition of influenza virus measured by antibody-absorption, J Exper Med *98*, 619-639

Jensen, K E, Minuse, E, and Ackermann, W W, 1955, Serologic evidence of American experience with newborn pneumonitis virus (Type Sendai), J Immunol *75*, 71-77

Jensen, K E, and Peterson, W D, Jr, 1957, Comparative measurements of antigenic differences among human and swine influenza viruses, J Immunol *78*, 365-372

Jordan, E O, 1927, Epidemic Influenza, a Survey, Chicago, Ill, Am Med Assoc, 599 pp

Kuroya, N., Ishida, M, and Shiratori, T., 1953, Newborn virus pneumonitis *(Type Sendai) I Clinical* observation of a new virus possessing hemagglutinin activity Yokohama Med Bull *4*, 217-233

Ledinko, N, 1955, Production of plaques with in-

[illegible]

Organ *8*, 683-723.

Lindenmann, J, Burke, D C, and Isaacs, A, 1957, Studies on the production, mode of action and *properties of interferon, Brit J Exper Path 38*, 551-562

Liu, C., 1955a, Studies on influenza infection in ferrets by means of fluorescein-labelled antibody I The pathogenesis and diagnosis of the disease J *Exper Med 101, 655-676*

———, 1955b, Studies on influenza infection in ferrets by means of fluorescein-labelled antibody II The role of soluble antigen in nuclear fluorescence and cross reactions J Exper Med *101*, 677-686

Maassab, H, and Francis, T, Jr, 1958, Unpublished material

MacCallum, W G, 1919, The pathology of the pneumonia in the United States Army camps during the winter of 1917-18 Monograph of Rock Inst for *Med Res, No 10, New York 147 pp*

McClelland, L, and Hare, R, 1941, Adsorption of influenza virus by red cells and a new *in vitro* method of measuring antibodies for influenza virus, Canad J Pub Health *32*, 530-538

Magill, T P, 1940, A virus from cases of influenza-like upper respiratory infection, Proc Soc Exper Biol & Med *45*, 162-164

———, 1945, Study of influenza in prisoner of war camp Unpublished data.

Magill, T P, and Francis, T, Jr, 1936, Antigenic differences in strains of human influenza virus, Proc. Soc Exper Biol & Med *35*, 463-466

———, 1938, Antigenic differences in strains of epidemic influenza virus, Brit J Exper Path *19*, *273-284*

[illegible]

Hartman, F W, Horsfall, F. L, Jr, and Kidd, [illegible]

M [illegible]

Med *74*, 651-653

Miller, G L, Lauffer, M A, and Stanley, W M, 1944, Electrophoretic studies on PR8 influenza virus, J Exper Med *80*, 549-559

Minuse, E, Quilligan, J J, Jr, and Francis, T, Jr, 1954, Type C influenza virus I Studies of the virus and its distribution J Lab & Clin Med *43*, 31-42

Morgan, C, Rose, H M, and Moore, D H, 1956, Structure and development of viruses observed in the electron microscope III Influenza virus J Exper Med *104*, 171-182

Mosley, V M., and Wyckoff, R W G, 1946, Electron micrography of virus of influenza, Nature, London *157*, 263

Mulder, J., 1957, Asiatic influenza in the Netherlands, Lancet *2*, 334

Mulder, J., and Masurel, N, 1958, The presence of pre-epidemic antibody against the 1957 strain of Asiatic influenza in the serum of older people living in the Netherlands In press

Mulder, J., and Verdonk, G J, 1949, Studies on the pathogenesis of a case of influenza-A pneumonia of three days' duration, J Path & Bact *61*, 55-61

Murphy, J S, and Bang, F B, 1952, Observations with the electron microscope on cells of the chick chorio-allantoic membrane infected with influenza virus, J Exper Med *95*, 259-268

Nishikawa, F., and Fukumi, H, 1954, Shape and size of hemagglutinating virus of mice (HUM), Jap J M Sc & Biol *7*, 513-522

Opie, E L., Blake, F G, Small, J C, and Rivers, T M., 1921, Epidemic Respiratory Disease The Pneumonias and Other Infections of the Respiratory Tract, Accompanying Influenza and Measles St Louis, Mosby 402 pp

Parker, F, Jr., Jolliffe, L S, Barnes, M W, and Finland, M., 1946, Pathologic findings in lungs of 5 cases from which influenza virus was isolated, Am J Path *22*, 797-819

Pearson, H E, 1944, Distribution of influenza virus Type A in infected eggs and the survival of virus under certain conditions of storage, J Bact *48*, 369-378

Pearson, H E, and Enders, J F, 1941, Cultivation of influenza A virus in roller tubes, Proc Soc Exper Biol & Med *48*, 140-143

Pfeiffer, R, 1892, Vorlaufige Mitteilungen uber die Erreger der Influenza, Deutsche med Wchnschr *18*, 28

Philip, R N., Bell, J A, Davis, D J, Beem, M O, and Beigelman, P M, 1954, Epidemiological studies on influenza in familial and general population groups I Preliminary report on studies with adjuvant vaccine Am J Pub Health *44*, 34-42

Quilligan, J J, Jr, and Francis, T, Jr, 1947, Serological response to intranasal administration of inactive influenza virus in children, J Clin Invest *26*, 1079-1087

Quilligan, J J, Jr, Minuse, E, and Francis, T, Jr, 1948, Homologous and heterologous antibody response of infants and children to multiple injections of a single strain of influenza virus, J Clin Invest *27*, 572-579

———, 1954, Type C influenza virus, II Intranasal inoculation of human individuals J Lab & Clin Med *43*, 43-47

Rickard, E R, and Francis, T, Jr, 1938, Demonstration of lesions and virus in lungs of mice receiving large intraperitoneal inoculations of epidemic influenza virus, J Exper Med *67*, 953-972

Salk, J E, 1948, Reactions to concentrated influenza virus vaccine, J Immunol *58*, 369-395

———, 1952, An interpretation of the significance of influenza virus variation for the development of an effective vaccine, Bull New York Acad Med *28*, 748-765

Salk, J E, Pearson, H E, Brown, P N, Smyth, C J, and Francis, T, Jr, 1945, Immunization against influenza with observations during an epidemic of influenza A one year after vaccination, Am J Hyg *42*, 307-322

Sano, T, Nutsu, I, and Nakagawa, I, 1953, Newborn virus pneumonitis (Type Sendai) I The clinical observation Tohoku J Exper Med *58*, 56

Sasahara, J, 1955, Studies on the HVJ (Hemagglutinating virus of Japan) newly isolated from the swine, Nat Inst of Animal Health, Tokyo, Report No 30, pp 34-38

Scadding, J G, 1937, Lung changes in influenza, Quart J Med *6*, 425-465

Schäfer, W, 1955, Vergleichende sero-immunologische Untersuchungen uber die Viren der Influenza and klassischen Geflugelpest Ztschr Naturforsch *10B*, 81-91

Sharp, D G, Taylor, A R, McLean, I W., Beard, D, and Beard, J W, 1944, Sedimentation velocity and electron micrographic studies of influenza viruses A (PR8 strain) and B (Lee strain) and the swine influenza virus, J Biol Chem *156*, 585-600

Shope, R E, 1931, Swine influenza III Filtration experiments and etiology J Exper Med *54*, 373-385

———, 1934, The infection of ferrets with swine influenza virus, J Exper Med *60*, 49-61

———, 1936, The influenzas of swine and man Harvey Lectures, Series 31, pp 183-213

———, 1938, Serological evidence for the occurrence of infection with human influenza virus in swine, J Exper Med *67*, 739-748

———, 1941a and b, The swine lungworm as a reservoir and intermediate host for swine influenza virus I The presence of swine influenza in healthy and susceptible pigs II The transmission of swine influenza virus by the swine lungworm J Exper Med *74*, 41-47, 49-68

———, 1943a and b, The swine lungworm as a reservoir and intermediate host for swine influenza virus III Factors influencing transmission of the virus and the provocation of influenza IV The demonstration of masked swine influenza virus in lungworm larvae and swine under natural conditions J Exper Med *77*, 111-126, 127-138

———, 1954, Ecology and virus reservoirs in Hartman, F W., Horsfall, F L, Jr, and Kidd, J G, (eds), The Dynamics of Virus and Rickettsial Infections, pp 125-141, New York, Blakiston

Smith, W, and Andrewes, C H, 1938, Serological races of influenza virus, Brit J Exper Path *19*, 293-314

Smith, W, Andrewes, C H, and Laidlaw, P P., 1933, A virus obtained from influenza patients, Lancet *2*, 66-68

Smorodintsev, A A, Gulamow, A G, and Tschalkina, O M, 1940, Über die spezifische Prophylaxe der epidemischen Grippe durch Inhalation antigrippischen Serums, Ztschr klin Med *138*, 756-765

Smorodintsev, A A, and Zhdanov, V M, 1957, Results and immediate tasks of the study of live influenza vaccine, Problems of Virology, *2*, 65

Sovinova, O, Tumova, B, Pouska, F, and Nemec, J, 1957, Isolation of virus causing the respiratory disease of horses, Cesk Epidem, Mikro, Immunol *6*, 213-220

American Medical Association, Council on Drugs, 1957, Antibiotics and influenza, J.A.M.A *165*, 58-59

Stone, J D, 1948, Prevention of virus infection with enzyme of *V cholerae* I Studies with viruses of

which enumerate infective and hemagglutinating virus particles, J Exper Med *100*, 135-161
Horsfall, F L, Jr, Lennette, E H, Rickard, E R, Andrewes, C H, Smith, W, and Stuart-Harris, C H, 1940b, The nomenclature of influenza, Lancet *2*, 413-414
Hoyle, L, and Fairbrother, R W, 1937, Antigenic structure of influenza viruses, the preparation of elementary body suspensions and the nature of the complement-fixing antigen, J Hyg *37*, 512-520
Hoyle, L, and Frisch-Niggemeyer, W, 1955, The disintegration of influenza virus particles on entry into the host cell Studies with virus labelled with radiophosphorus, J Hyg *53*, 474-486
Hubble, D, and Osborn, G R, 1941, Acute bronchiolitis in children, Brit M J *1*, 107-110
Isaacs, A, and Andrewes, C H, 1951, The spread of influenza evidence from 1950-1951, Brit M J *2*, 921-927
Isaacs, A, Depoux, R, and Fiset, P, 1954, The viruses of the 1952-3 influenza epidemic, Bull World Health Organ *11*, 967-979
Ishida, N, 1957 [Discussion new respiratory and ocular viruses], Ann New York Acad Sc *67*, 299-300
Ivánovics, G, Horváth, S, and Szollosy, E, 1954, The influenza virus adsorbing capacity of the vascular endothelium of various mammals Acta Microbiologica Acad Scient Hung *2*, 121-129
Jensen, K E, 1956, Influenza *in* Diagnostic Procedures for Virus and Rickettsial Disease, pp 241-262, New York, Am Public Health Assoc
———, 1957, The nature of serological relationships among influenza viruses *in* Advances in Virus Research, vol 4, pp 279-310, New York, Acad Press
Jensen, K E, Davenport, F M, Hennessy, A V, and Francis, T, Jr, 1956, Characterization of influenza antibodies by serum absorption, J Exper Med *104*, 199-209
Jensen, K E, and Francis, T, Jr, 1953, The antigenic composition of influenza virus measured by antibody-absorption, J Exper Med *98*, 619-639
Jensen, K E, Minuse, E, and Ackermann, W W, 1955, Serologic evidence of American experience with newborn pneumonitis virus (Type Sendai), J. Immunol *75*, 71-77
Jensen, K E, and Peterson, W D, Jr, 1957, Comparative measurements of antigenic differences among human and swine influenza viruses, J Immunol *78*, 365-372
Jordan, E O, 1927, Epidemic Influenza, a Survey, Chicago, Ill, Am Med Assoc, 599 pp
Kuroya, N, Ishida, M, and Shiratori, T, 1953, Newborn virus pneumonitis (Type Sendai) I Clinical observation of a new virus possessing hemagglutinin activity Yokohama Med Bull *4*, 217-233
Ledinko, N, 1955, Production of plaques with in- [illegible]
[illegible]
Med *72*, 233-240
Lepine, P, 1953, Méthodes de laboratoire appliquees à l'etude du virus grippal, Bull. World Health Organ *8*, 683-723.
Lindenmann, J, Burke, D C., and Isaacs, A, 1957, Studies on the production, mode of action and properties of interferon, Brit J Exper Path *38*, 551-562
Liu, C, 1955a, Studies on influenza infection in ferrets by means of fluorescein-labelled antibody I The pathogenesis and diagnosis of the disease J Exper Med *101*, 655-676
———, 1955b, Studies on influenza infection in ferrets by means of fluorescein-labelled antibody II The role of soluble antigen in nuclear fluorescence and cross reactions J. Exper Med *101*, 677-686
Maassab, H, and Francis, T, Jr, 1958, Unpublished material
MacCallum, W G, 1919, The pathology of the pneumonia in the United States Army camps during the winter of 1917-18 Monograph of Rock Inst for Med Res, No *10*, New York *147* pp
McClelland, L, and Hare, R, 1941, Adsorption of influenza virus by red cells and a new *in vitro* method of measuring antibodies for influenza virus, Canad J Pub Health *32*, 530-538
Magill, T P, 1940, A virus from cases of influenza-like upper respiratory infection, Proc Soc Exper Biol & Med *45*, 162-164
———, 1945, Study of influenza in prisoner of war camp Unpublished data
Magill, T P, and Francis, T, Jr, 1936, Antigenic differences in strains of human influenza virus, Proc Soc Exper Biol & Med *35*, 463-466
———, 1938, Antigenic differences in strains of epidemic influenza virus, Brit J Exper Path *19*, 273-284
von Magnus, P, 1947, Studies on interference in experimental influenza I Biological observations Arch Kemi, Mineral och Geol *24B*, no 7, 6 pp
———, 1954, "Incomplete" forms of influenza virus *in* Hartman, F W, Horsfall, F L, Jr, and Kidd, J G, (eds), The Dynamics of Virus and Rickettsial Infections, pp 36-44, New York, Blakiston
Merchant, D J, and Morgan, H R, 1950, Inhibition of the phagocytic action of leucocytes by mumps and influenza viruses, Proc Soc Exper Biol & Med *74*, 651-653
Miller, G L, Lauffer, M A, and Stanley, W M, 1944, Electrophoretic studies on PR8 influenza virus, J Exper Med *80*, 549-559
Minuse, E, Quilligan, J J, Jr, and Francis, T, Jr, 1954, Type C influenza virus I Studies of the virus and its distribution J Lab & Clin Med *43*, 31-42
Morgan, C, Rose, H M, and Moore, D H, 1956, Structure and development of viruses observed in the electron microscope III Influenza virus J Exper Med *104*, 171-182
Mosley, V M, and Wyckoff, R W G, 1946, Electron micrography of virus of influenza, Nature, London *157*, 263
Mulder, J, 1957, Asiatic influenza in the Netherlands, Lancet *2*, 334
Mulder, J, and Masurel, N, 1958, The presence of pre-epidemic antibody against the 1957 strain of Asiatic influenza in the serum of older people living in the Netherlands. In press

Mulder, J., and Verdonk, G J, 1949, Studies on the pathogenesis of a case of influenza-A pneumonia of three days' duration, J Path & Bact *61*, 55-61

Murphy, J S., and Bang, F B, 1952, Observations with the electron microscope on cells of the chick chorio-allantoic membrane infected with influenza virus, J. Exper Med *95*, 259-268.

Nishikawa, F., and Fukumi, H., 1954, Shape and size of hemagglutinating virus of mice (HUM), Jap J M Sc & Biol *7*, 513-522

Opie, [redacted] L., Blake, F G., Small, J C., and Rivers, T M., 1921, *Epidemic Respiratory Disease* The Pneumonias and Other Infections of the Respiratory Tract, Accompanying Influenza and Measles St Louis, Mosby 402 pp

Parker, F, Jr., Jolliffe, L S., Barnes, M W., and Finland, M., 1946, Pathologic findings in lungs of 5 cases from which influenza virus was isolated, Am J Path *22*, 797-819

Pearson, H E., 1944, Distribution of influenza virus Type A in infected eggs and the survival of virus under certain conditions of storage, J Bact *48*, 369-378

Pearson, H E, and Enders, J F, 1941, Cultivation of influenza A virus *in* roller tubes, Proc Soc Exper Biol [redacted] Med *48*, 140-143

Pfeiffer, R, 1892, Vorlaufige Mitteilungen uber die Erreger der Influenza, Deutsche med Wchnschr *18*, 28

Philip, R N., Bell, J A., Davis, D J., Beem, M O., and Beigelman, P M., 1954, Epidemiological studies on influenza in familial and general population groups I Preliminary report on studies with adjuvant vaccine Am J Pub Health *44*, 34-42

Quilligan, J J., Jr., and Francis, T, Jr., 1947, Serological response to intranasal administration of inactive influenza virus in children, J Clin Invest *26*, 1079-1087

Quilligan, J J, Jr, Minuse, E, and Francis, T, Jr, 1948, Homologous and heterologous antibody response of infants and children to multiple injections of a single strain of influenza virus, J Clin Invest *27*, 572-579

———, 1954, Type C influenza virus, II Intranasal inoculation of human individuals J Lab & Clin Med *43*, 43-47

Rickard, E R, and Francis, T, Jr, 1938, Demonstration of lesions and virus in lungs of mice receiving large intraperitoneal inoculations of epidemic influenza virus, J Exper Med *67*, 953-972

Salk, J E, 1948, Reactions to concentrated influenza virus vaccine, J Immunol *58*, 369-395

———, 1952, An interpretation of the significance of influenza virus variation for the development of an effective vaccine, Bull New York Acad Med *28*, 748-765

Salk, J E, Pearson, H E, Brown, P N, Smyth, C J, and Francis, T, Jr, 1945, Immunization against influenza with observations during an epidemic of influenza A one year after vaccination, Am J Hyg *42*, 307-322

Sano, T, Nutsu, I, and Nakagawa, I, 1953, Newborn virus pneumonitis (Type Sendai) I The clinical observation Tohoku J Exper Med *58*, 56

Sasahara, J, 1955, Studies on the HVJ (Hemagglutinating virus of Japan) newly isolated from the swine, Nat Inst of Animal Health, Tokyo, Report No 30, pp 34-38

Scadding, J G, 1937, Lung changes in influenza, Quart J Med *6*, 425-465

Schafer, W, 1955, Vergleichende sero-immunologische Untersuchungen uber die Viren der Influenza and klassischen Geflugelpest Ztschr Naturforsch *10B*, 81-91

Sharp, [redacted] G, Taylor, A R, McLean, I W, Beard, D, and Beard, J W, 1944, Sedimentation velocity and electron micrographic studies of influenza viruses A (PR8 strain) and [redacted] (Lee strain) and the swine influenza virus, J Biol Chem *156*, 585-600

Shope, R E, 1931, Swine influenza III Filtration experiments and etiology J Exper Med *54*, 373-385

———, 1934, The infection of ferrets with swine influenza virus, J Exper Med. *60*, 49-61

———, 1936, The influenzas of swine and man Harvey Lectures, Series 31, pp 183-213

———, 1938, Serological evidence for the occurrence of infection with human influenza virus in swine, J Exper Med *67*, 739-748

———, 1941a and b, The swine lungworm as a reservoir and intermediate host for swine *influenza* virus I The presence of swine influenza in healthy and susceptible pigs II The transmission of swine influenza virus by the swine lungworm J Exper Med *74*, 41-47, 49-68

———, 1943a and b, The swine lungworm as a reservoir and intermediate host for swine influenza virus. III Factors influencing transmission of the virus and the provocation of influenza IV The demonstration of masked swine influenza virus [redacted] lungworm larvae and swine under natural conditions J Exper Med *77*, 111-126, 127-138

———, 1954, Ecology and virus reservoirs in Hartman, F W., Horsfall, F L, Jr, and Kidd, J G, (eds), The Dynamics of Virus and Rickettsial Infections, pp 125-141, New York, Blakiston

Smith, W, and Andrewes, C H, 1938, Serological races of influenza virus, Brit J Exper Path *19*, 293-314

Smith, W, Andrewes, C H, and Laidlaw, P P, 1933, A virus obtained from influenza patients, Lancet *2*, 66-68

Smorodintsev, A A, Gulamow, A G, and Tschalkina, O M, 1940, Über die spezifische Prophylaxe der epidemischen Grippe durch Inhalation antigrippischen Serums, Ztschr klin Med *138*, 756-765

Smorodintsev, A A, and Zhdanov, V M, 1957, Results and immediate tasks of the study of live influenza vaccine, Problems of Virology, *2*, 65

Sovinova, [redacted], Tumova, B, Poustka, F, and Nemec, J, 1957, Isolation of virus causing the respiratory disease of horses, Cesk Epidem, Mikro, Immunol *6*, 213-220

American Medical Association, Council on Drugs, 1957, Antibiotics and influenza, J A M A *165*, 58-59

Stone, J D, 1948, Prevention of virus infection with enzyme of *V cholerae* I Studies with viruses of

mump-influenza group in chick embryo Australian J Exper Biol & M Sc 26, 49-64

Straub, M, 1937, Microscopical changes in lungs of mice infected with influenza virus, J Path & Bact 45, 75-78

Straub, M, and Mulder, J, 1948, Epithelial lesions in the respiratory tract in human influenzal pneumonia, J Path & Bact 60, 429-434

Stuart-Harris, C H, 1939, A neurotropic strain of human influenza virus, Lancet 1, 497-499

———, 1953, Influenza and other virus infections of the respiratory tract, London, Arnold, 235 pp

Stuart-Harris, C H, Franks, Z, and Tyrrell, D, 1950, Deaths from influenza—a statistical and laboratory investigation, Brit M J 1, 263-266

Taylor, R M, 1941, Experimental infection with *influenza A virus in mice The increase of intra*pulmonary virus after inoculation and the influence of various factors thereon J Exper Med 73, 43-55

———, 1949, Studies on survival of influenza virus between epidemics and antigenic variants of the virus, Am J Pub Health 39, 171-178

———, 1951, A further note on 1233 ("Influenza C") virus, Arch ges Virusforsch 4, 485-500

Thompson, T, 1852, Annals of Influenza, pp 6-10, London, The Sydenham Soc, 406 pp

Thomson, D, and Thomson, R, 1933, Influenza (part 1), with special reference to the part played by Pfeiffer's bacillus, streptococci, pneumococci, etc, and the virus theory Ann Pickett-Thomson Research Lab 9, 1-640

———, 1934, Influenza (part 2), with special reference to the complications and sequelae, bacteriology of influenzal pneumonia, pathology, epidemiological data, prevention and treatment Ann Pickett-Thomson Research Lab 10, 641-1557

Tyrrell, D A J, 1954, Separation of inhibitors of hemagglutination and specific antibodies for influenza viruses by starch zone electrophoresis, J Immunol 72, 494-502

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van der Veen, J, and Mulder, J, 1950, Studies on the antigenic composition of human influenza virus A strains with the aid of the haemagglutina*tion inhibition technique Thesis, Leiden, Holland*

Vogel, J, and Shelokov, A, 1957, Adsorption-hemagglutination test for influenza virus in monkey kidney tissue culture, Science 126, 358-359

Wang, C I, 1948, The relation of infectious and hemagglutination titers to the adaptation of influenza virus to mice, J Exper Med 88, 515-519

Watson, B K, and Coons, H A, 1954, Studies of

W

Conn, Yale Univ Press, 61 pp

Wyckoff, [redacted] W G, 1951, *Electron microscopy of* chick embryo membrane infected with PR8 in influenza, Nature, London *168*, 651-652

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32

Smallpox, Cowpox and Vaccinia

INTRODUCTION

Smallpox has been recognized as a disease entity for many centuries. Because of its striking clinical picture of severe illness associated with a pustular eruption, its occurrence in epidemics with high mortality and the dread which it has engendered in populations exposed to its ravages, the records of its incidence over the past few hundred years are more nearly complete than in the case of any other infectious disease. Although smallpox is not as widespread as it was a hundred years ago, there are still areas in the world where the infection is endemic, and constant vigilance is necessary for its control. In the present century a milder form of smallpox, with a very low mortality, has become common in Africa, Western Europe and America although such a type was recorded in England in the 18th century (Creighton, 1894). For this relatively benign disease the names "variola minor" or "alastrim" are used. It is clinically and epidemiologically distinct from the more severe form known as "variola major" or "classic smallpox."

Smallpox was the first disease for which prophylaxis by active immunization was widely practiced. In the 18th century this was done by the inoculation of smallpox (variolation). As a consequence of Jenner's paper, published in 1798, variolation was superseded by the inoculation of cowpox, a relatively mild infection of cows which occasionally affected farm workers. For many years the inoculation of cowpox or vaccination was carried on by arm-to-arm passage, and occasionally fresh strains were introduced from infected cows, so that as early as 1868 Ballard records that it was uncertain whence vaccine lymphs in current use were derived. For more than 60 years the virus strains used in vaccine lymph establishments throughout the world have been passed by dermal inoculation in calves, sheep or rabbits. During this time some strains at least have altered in certain minor characteristics so that they now differ from virus strains recently isolated from natural cowpox infections. These vaccinia strains have been the subject of extensive laboratory study, and to avoid confusion the strains recently isolated from cowpox will be referred to in this chapter as cowpox virus. Tentative approval has recently been given to the following names for the viruses under consideration: *Poxvirus variolae* (smallpox), *Poxvirus bovis* (cowpox) and *Poxvirus officinale* (vaccinia).

SMALLPOX

(SYNONYMS: Variola, *petite vérole*, *Blattern*)

HISTORY

It is generally stated that smallpox was prevalent in India and China before the Christian era but its first appearance in epidemic form further west was apparently in Arabia in the 6th century, possibly brought there from Africa by an Abyssinian Army. The

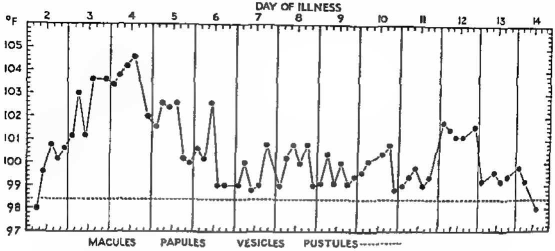

FIG 112. Chart from a fatal case of smallpox in an unvaccinated adult-death on 14th day of illness

disease was described by Rhazes in the 10th century, in the introduction to his work, translated from Arabic by Greenhill and printed in 1848, Rhazes states that the disease was described by Galen in the 2nd century Dissemination of smallpox from Asia to Europe and North Africa was associated with the invasion of these continents by the Saracens According to Creighton, the first clear account of smallpox in Britain appears early in the 16th century At this time also the disease was common among African natives brought by slave ships to the West Indies, and subsequently the disease took terrible toll among the Mexicans. In England smallpox was most deadly as an endemic

mortality in some years was approximately one sixth of the birth rate Over 90 per cent of the cases occurred in children under 10 years of age and in Chester from 1772 to 1777 one third of all deaths in this age group was caused by smallpox (Haygarth, 1793). In America where epidemics occurred at infrequent intervals the disease affected persons of all ages In Boston, Mass, there was no serious epidemic of smallpox between 1730 and 1752 In the latter year over one third of the population suffered from it, and all ages were attacked (Creighton, 1894). The endemic prevalence in Britain may have been maintained in part by the practice of variolation which was widespread in the second half of the 18th century Variolation was finally suppressed in England by Act of Parliament in 1840

The inoculation of smallpox introduced in England in 1721 did not immediately become popular, for the practice was not without risk to the patient The inoculation of smallpox matter by incision or puncture of the skin usually produced a local lesion with fever commencing on the 7th or the 8th day and a general eruption on the 9th or the 10th While this inoculated smallpox had a shorter incubation period and was generally milder than the naturally acquired disease, this was not always so In the first 8 years of the practice of variolation in England 17 persons of 897 inoculated were believed to have died from the subsequent disease (Woodville, 1796). Therefore, the practice fell into disfavor, but interest was revived following its successful use in Charlestown, S C, in 1738 There the practice was introduced of using matter from inoculated persons for further inoculation, and this modification was introduced into England, where inoculation was again taken up Most Western European countries did not adopt variolation until after 1750. With more careful selection of the material for inoculation and better care of the patient there was less risk associated with the practice than in the years immediately after its introduction However, even up to the time of Jenner's publication in 1798, the death rate from inoculated smallpox was reported to be about 1:200 to 1:500, at this period the mortality from naturally acquired smallpox was 10 to 20 per cent. It was under-

FIG 113 Pustular eruption of smallpox in an unvaccinated child

stood as early as 1722 that the inoculated disease was contagious, but not until many years later was the practice advocated, and in some instances followed, of inoculating whole communities at one time and isolating from the inoculated those who had not had the smallpox

In the 19th century the incidence of smallpox declined in Western countries and, except in such epidemic years as 1871 and 1872, became less important than scarlet fever and measles as a cause of death in childhood

In the 20th century variola major has maintained its lethal qualities in those areas of the world where it is endemic for example in 1950 there were recorded in India 157,322 cases with 41,092 deaths (Murray, 1951) On the other hand, the mortality from variola minor has been low Between 1920 and 1930, years of high incidence of variola minor in the United States and Britain, the mortality was less than 1 per cent

Clinical Picture

Little has been added by later writers to Rickett's classic description of the clinical aspects of smallpox (Ricketts and Byles, 1908), the following brief account conforms with his teaching, and the remarks on alastrim are largely based on the analysis of over 13,000 cases seen by Marsden between 1928 and 1934 (Marsden, 1948).

The incubation period generally lasts for 12 days The clinical illness begins with an initial toxemic phase which lasts for 4 to 6 days and is characterized by fever, constitutional symptoms and prostration There is usually severe headache, backache, pains in the limbs and sometimes vomiting In a proportion of cases a toxemic or prodromal rash with a predilection for the groins, the axillae and the flanks is seen, in milder cases this may be erythematous or in more severe cases petechial, and in the worst cases petechial hemorrhages may be present over much of the body surface On the third or fourth day the focal rash occurs and within a day or two thereafter the temperature falls near to normal, and the patient feels much better The focal rash usually appears first on the buccal and the pharyngeal mucosa, the face or the forearms and the hands and then spreads to the trunk and the lower limbs, so that for the first few days of the eruption the lesions on the face and the arms may be a day in advance of those on the legs In the typical case, however, the lesions in any one area are all at the same stage of development The macular rash quickly becomes papular, and within 2 or 3 days the papules have developed into vesicles, this stage is quickly followed by pustulation After about 8 or 9 days from the appearance of the focal rash crusting begins and is usually complete in 14 to 16 days from the onset of the illness By the end of 3 weeks most of the crusts have separated with the exception of those embedded in the palms of the hands and the soles of the feet The eruption is usually most profuse on the face, which may be swollen and edematous, and on forearms and hands The trunk generally shows more pocks on the upper part of

the back than on the chest Lesions are still less on the abdomen, while the groins and the axillae may be relatively free. More lesions are seen on the feet and the ankles and below the knee than on the thighs Except when the eruption is sparse lesions are to be found on the palms of the hands and the soles of the feet There is in all except the mildest cases some recurrence of fever associated with the stage of pustulation, but in cases which recover the temperature becomes normal as the lesions begin to dry up

In the most severe cases of smallpox the patient may die within the first week, even before the focal rash appears, for in some of these the focal rash is delayed The patient is extremely toxemic, and from the second or the third day hemorrhages occur in the skin, and there may be bleeding into the conjunctiva and from the mouth, the nose, the vagina and the bowel—*purpura variolosa* Hemorrhages may appear after the eruption in some toxic cases The extravasations of blood are situated in the corium below the epidermal lesions which appear hemorrhagic, but in addition hemorrhages may occur between the pustules—*variola pustulosa hemorrhagica* The severity of the illness in the eruptive phase parallels the extent of the skin eruption

Where the eruption is confluent on the face and the arms the vesicles may feel soft and show marked umbilication, this is especially so in the type of case which Dixon classifies as malignant confluent (Dixon, 1948) In modified cases with a discrete rash the elements are round, raised and tense, and depression of the center only appears, if at all, as the lesions are drying up In the mildest type of clinical infection the usual initial illness may not be followed by any focal eruption—*variola sine eruptione.* In vaccinated contacts a febrile illness without an eruption may be associated with radiologic evidence of pulmonary involvement The variolous nature of some of these febrile illnesses without eruption in smallpox contacts can be established by modern laboratory methods.

In cases modified by vaccination the prodromal illness may be severe. The rash may be scanty, or it may be profuse but superficial and evolving rapidly. These modified cases of variola major may be indistinguishable clinically from cases of variola minor; but unvaccinated persons infected through contact with them suffer from unmodified variola major.

In alastrim there is essentially the same clinical picture as in variola major. The prodromal or pre-eruption illness may be quite severe, but hemorrhagic or toxic cases are rare. Marsden (1948) records only 3 among 13,686 patients, and 2 of these died In only 19 was the eruption confluent The lesions of the focal rash in alastrim tend to be more superficial and develop more rapidly, so that the eruptive phase of the illness tends to be shorter than in classic smallpox In many cases of alastrim there may be no secondary fever during the pustular stage.

The pregnant woman seems to fare particularly badly when she contracts smallpox Abortion is common because of the tendency to uterine hemorrhage which the toxemia engenders. The fetus may escape this risk but acquire infection in utero at the time of onset of illness in the mother when viremia is present. When infection takes place near the end of pregnancy the child may develop clinical disease within a few days after birth, or infection may be acquired at the time of birth if the disease is still active in the mother. A few rare instances have been recorded of smallpox in babies who were infected in utero a few days before birth, although the mother showed no clinical signs of disease (Bancroft, 1904, Lynch, 1932)

The patients who die in the first week of illness often show signs of edema of the lungs due to heart failure, and terminal pneumonia may be present But in some of these cases there may be no signs of bacterial infection, and in these death is due to the toxic effects of an overwhelming virus infection However, the majority of deaths, occur in the pustular stage toward the end of the second week An encephalitis with severe perivascular demyelination similar to that occasionally associated with vaccination and with measles may occur between the 8th and the 15th day of disease as an uncommon complication in both variola major and minor (Marsden, 1948).

The variation in mortality in different clinical types of variola major infection are well illustrated by the figures from the Minneapolis outbreak in 1924 and 1925 recorded by

Sweitzer and Ikeda (1927) Among the 1,430 cases the over-all mortality was 25 5 per cent Of 581 cases admitted to one hospital, 246 died This hospital group was subdivided as follows 10 unclassified type, no deaths, 225 discrete type, 14 deaths, 151 confluent type, 68 deaths; 144 hemorrhagic type, 113 deaths, 51 purpuric type, 51 deaths A similar mortality rate in relation to clinical types was recorded by Dixon (1948) in 500 cases studied in an outbreak in Tripolitania in 1946

Pathology

The site of entry of the virus is believed to be in the upper respiratory tract, and during the incubation period it probably multiplies in lymphoid and other internal tissues The patient is not infectious during the incubation period, so that it is unlikely that there is an open lesion in the respiratory mucosa At or just before the onset of illness there is viremia and from the circulation the virus localizes in the skin, the mucous membranes and other tissues where lesions are shortly to appear Soon after the focal skin eruption appears the patient's condition improves, the temperature drops, and neutralizing antibodies become detectable in the serum By then, the virus has multiplied within the epithelial cells in the focal lesions in the skin, and scrapings made at this time show enormous numbers of elementary bodies in appropriately stained smears Virus within cells is protected from the action of antibody so that destruction of cells goes on with consequent inflammatory changes The secondary rise of temperature associated with the stage of pustulation is probably due to the absorption of the toxic products of widespread cell necrosis

Little has been added to our knowledge of the histology of the specific lesions in the skin and the mucous membranes since the classic work of Councilman et al (1904) Their findings have been, for the most part confirmed by recent studies on material from 177 fatal cases of variola major (Bras 1952) The histology of the skin lesions in alastrim, based on the examination of 25 skin biopsies from 18 cases of alastrim at various stages of the disease, have been described by de Jong (1956) The earliest change to be observed in the skin is dilatation of the capillaries in the papillary layer of the corium with swelling of the lining endothelium There is a perivascular infiltration with lymphocytes and histocytes Changes then appear in the overlying epidermis At first a few cells in the middle layer of the epidermis become enlarged, and vacuoles appear in the cytoplasm which stains poorly The nucleus condenses and later disappears There is proliferation of cells in the malphigian layer and this, together with the swelling of cells, leads to great thickening of the epithelial layer As the process of degeneration spreads to neighboring cells in the middle layer there is intercellular edema, the cell membranes rupture and a vesicle is formed The vesicle tends to be loculated with septa formed by the remains of incompletely destroyed cells The epithelial cells beneath the vesicle are at first compressed and disorderly, but individual cells or groups of cells get detached and become rounded off The nuclei shrink, and the cytoplasm becomes more eosinophilic In the fully developed vesicle the roof is formed of compressed cells of the stratum spinosum, the keratohyalin layer and the horny layer and is very thin over the summit of the vesicle The base is formed of degenerate rounded cells of the malphigian layer which may later disappear, so that the pock cavity extends to the corium Pustules form when polymorphonuclear leukocytes enter the vesicle from the dermis, and the septa which previously crossed the vesicle disappear In the healing stage fluid is absorbed from the pustule and the contents dry up Epithelial cells from the sides of the cavity encroach upon it and proceed to grow under the residual mass of the exudate so that finally a crust is left of degenerated epithelial cells, leukocytes and debris The crusts separate in the 3rd week of illness The pitting, which is for the most part confined to the face even in severe cases, is caused, according to Bras (1952) by destruction of sebaceous glands followed by organization and subsequent shrinking of granulation tissue In cases of purpura variolosa extravasated blood cells are present in the corium, and even when no skin lesions have been obvious to the naked eye at the time of death, small areas of degeneration with commencing vesicle formation are to be seen in the epidermis microscopically Guarnieri bodies may be found in the swollen epithelial cells

In the mucous membrane of the pharynx, the uvula, the cheeks, the tongue, the larynx and the upper part of the esophagus and the trachea, lesions are to be found concurrently with those in the skin In hemorrhagic cases there may be associated hemorrhages in the submucosa No vesicles are formed, but necrosis of the epithelium occurs in small focal

lesions or may involve extensive areas of the mucosa. Because of the absence of an impervious keratinized layer the damaged epithelial cells are shed from these lesions at an early stage in the disease so that the patient first becomes infective from this source. Around the necrosed cells there is congestion and fibrinous and polymorphonuclear exudate; very soon the necrosed tissue sloughs off, leaving mucosal defects. These finally heal by growth of new epithelium and without scarring Small areas of necrosis are sometimes to be found in lymphoid tissue in the submucosa in the pharynx, the tonsils and the paratracheal lymph glands. Within the sinusoids of the liver, the sinuses of the spleen and the lymph nodes and the capillaries in the kidney and other organs large mononuclear basophilic cells are constantly present; these cells are regarded by Bras (1952) as large *lymphocytes and plasma cells having* their origin in the spleen and the lymph glands Focal interstitial accumulation of these cells in various organs is a constant and characteristic feature In the bone marrow neutrophilic polynuclear cells are scarce, and there is reactive proliferation of reticulum cells *Sometimes the marrow is normoblastic*

In the heart, apart from subendocardial hemorrhages and occasional collection of mononuclear cells around small vessels, pathologic changes are infrequent Councilman et al. (1904) commonly found bronchitis and bacterial bronchopneumonia, and Sweitzer and Ikeda *(1927) considered bronchopneumonia* as the cause of death in their 9 patients coming to autopsy On the other hand, in Bras's (1952) series of 177 autopsies, although hyperemia was common in the lungs, in only 25 was bronchopneumonia sufficiently developed to be considered as the cause or concurrent cause of death The fact that in Bras's series, nearly all the patients had had antibiotic treatment probably accounts for the difference in the findings.

The liver is constantly enlarged, but apart from occasional small hemorrhages gross changes are not obvious. The endothelial cells lining the sinusoids are frequently swollen and proliferating, and in the portal spaces there is an accumulation of lymphocytes with a few plasma cells and large mononuclears

The spleen is usually larger than normal and congested. Microscopically, the follicles appear to be hyperplastic, and in the pulp few neutrophilic granulocytes are to be seen. There is evidence of proliferation of reticulum cells, and numerous large lymphoid cells are present. In the kidneys some dilatation of the vessels and degeneration of epithelial cells is to be found, especially in the outer pyramidal zone. Focal necrotic lesions were frequently observed in the testes by Councilman et al (1904), and Bras (1952) noted that focal lesions, not readily detectable with the naked eye, were most prominent in patients dying during the pustular stage of the disease.

Cytoplasmic inclusions are characteristic of infection with variola and vaccinia viruses They may be demonstrated in various tis-

affected skin and mucous membranes. Early in the disease, in sections stained with hematoxylin and eosin, these inclusions appear as round or oval homogeneous faintly basophilic *or acidophilic masses (Guarnieri bodies)* lying in the cytoplasm, usually close to the nucleus. There may be one or more to a cell, and each is usually surrounded by a clear unstained halo These bodies are typically seen in the corneal epithelium of animals infected with variola or vaccinia viruses In *older lesions, however, both in variola* and in the skin of the rabbit infected with vaccinia the inclusions have a granular appearance, an irregular outline and may occupy a large part of the cytoplasm of infected cells (Downie and Dumbell, 1947) The appearance of the cytoplasmic inclusion varies not *only with the stage of infection* but with the methods of fixation and staining used in the preparation of sections Goodpasture et al (1932) concluded that the acidophilic cytoplasmic inclusions seen in sections were produced, in part at least, by the heaping up of masses of virus particles. The observations of Himmelweit (1938) on the development of vaccinia in the chorio-allantois, the tissue culture studies of Bland and Robinow (1939) and recent work with the electronmicroscope [illegible] that [illegible] masses [illegible] mount

of matrix. Intranuclear inclusions have been described in the lesions of smallpox in man and monkeys (Magrath and Brinckerhoff, 1904; Torres, 1935-36) but they are not a conspicuous feature of the lesions. They are not mentioned by Bras (1952) but have been found by the writer in sections of the skin from 2 of 9 fatal cases of variola major ex*amined They have not been identified in* infected chorio-allantois The relationship of

these intranuclear inclusions to the virus is unknown and may be independent of the virus itself. Differences in the staining properties of the inclusions in variola major and variola minor reported by Torres (1935-36) have not been observed by de Jong (1956)

In older studies on smallpox the importance of bacterial infection as a cause of death was emphasized, and the morbid anatomy was complicated by evidence of superadded infection with streptococci and other organisms Recent observations indicate that although bacterial infection and particularly bronchopneumonia may contribute to a fatal issue, it is not an essential feature of the pathology, nor is it responsible for the high mortality in variola major Although antibiotic treatment was used in the outbreaks in Britain during the years 1949-53, there were 32 deaths among 115 cases (mortality 28%) Blood cultures made before death from fulminating cases frequently fail to reveal bacteria, and cultures from unbroken pustules are usually bacteriologically sterile The polymorphonuclear exudate in skin pustules and in the lesions of mucous membranes is, as Councilman et al (1904) believed, secondary to cell necrosis due to the specific action of the virus.

Experimental Infection, Host Range

The infection which resulted from the inoculation of smallpox matter into human skin during the period of variolation in Europe and America has been mentioned in an earlier section The monkey appears to be the only animal other than man to contract variola under natural conditions An epidemic of smallpox in Brazil is reported to have been associated with an epizootic among Mycetes and Cebus monkeys in the area, the bodies of sick and dead animals were covered with variolous pustules (Blaxall, 1930) An orangutan (*Simia satyrus*) which lived in the zoo and contracted smallpox during an outbreak in Djakarta in 1951 is mentioned by Bras (1952)

Experimental inoculation of variolous material into the skin of monkeys results in a typical local lesion followed in some animals by a rise in temperature on the 6th and an exanthema on the 8th day (Brinckerhoff and Tyzzer, 1906) General disease may follow inhalation of dried variolous exudate or inoculation by scarification of the tracheal mucosa and less commonly after inoculation on the mucosa of the nose, the lip or the palate

Other animal species are only slightly susceptible to variola virus The work on this subject has been confused by the fact that experiments have usually been carried out in laboratories engaged in work with vaccinia virus to which animals, notably the rabbit and the calf, are highly susceptible. As a result many reports of so-called primary isolation of variola virus in animals and its subsequent transformation to vaccinia during passage are now discounted Inoculation of fluid from smallpox pustules on the cornea of the rabbit frequently produces a keratitis with the formation of typical Guarnieri bodies in affected corneal epithelium, this is the basis of Paul's test which has been used as a diagnostic procedure After intracutaneous injection of variolous material in the rabbit a local lesion results similar to that produced by vaccinia virus, but in contrast with vaccinia, attempts at continued propagation in the skin fail. Variola virus may be carried through several passages by intratesticular inoculation in the rabbit, but it shows no tendency to increase in virulence or to give rise to a vaccinial variant (Nelson 1943, Downie and Dumbell, 1956) On intranasal inoculation of mice variola virus is less virulent than vaccinia and produces only minimal lesions in the lungs (Nelson, 1939)

The virus of smallpox grows well on the chorio-allantois of developing chick embryos, producing typical small grayish white pock-like lesions which do not appreciably alter in successive passages (Torres and Teixeira, 1935, Lazarus et al, 1937, Buddingh, 1938) The pocks are smaller, more convex and have less tendency to necrosis than those caused by most strains of vaccinia virus and can be readily distinguished from them (For illustrations see Nelson, 1939, and Downie and Dumbell, 1947) If the titer of the inoculum is low, the chick embryo may survive and the lesions on the chorio-allantois heal, but with larger doses, even in early passages, death of the embryo occurs on the 3rd or the 4th day after inoculation (Smadel, 1952, Helbert, 1957). The virus from alastrim cases has in general the same low virulence for animals as has the virus from variola major However, Dinger (1956) has found that variola major

virus may be recovered in greater quantity than variola minor strains from 4 to 6 days after inoculation of comparable infecting doses on the chorio-allantois This may be related to the greater virulence for chick embryos of variola major strains reported by Helbert (1957)

Etiology

The variola virus has not been studied nearly as intensively as the closely related vaccinia virus Until the development of techniques using the chick embryo, human cases of smallpox or experimentally infected monkeys were the only sources of material for study, and monkeys were the only suitable experimental animals The chick embryo chorio-allantois has provided a readily available medium for studies during the past 20 years

The virus of variola was first demonstrated microscopically in variolous lymph by Buist in 1887 (Gordon, 1937) From their appearance in stained preparations he estimated the virus particles to be about 0 15 μ in diameter The bodies were again described by Paschen in 1906. He believed that these minute bodies were the virus and that their demonstration in stained smears from lesions in the skin and mucous membranes was of diagnostic value in smallpox Subsequent work has confirmed Paschen's view The elementary bodies are small spherical structures having a diameter of approximately 200 mμ They can be demonstrated in large numbers in smears from the early skin lesions of smallpox by staining with aniline dyes or by silver impregnation methods They are readily visible by darkground illumination or by phase contrast microscopy By electronmicroscopy the virus particles in smallpox crust extract and in smallpox vesicle fluid are indistinguishable from the elementary bodies of vaccinia virus (Nagler and Rake, 1948, van Rooyen and Scott, 1948)

Variola virus is quite stable In exudates from smallpox cases the virus has long been known to be resistant to drying Dried powdered crusts were used in China to produce immunizing infections, and matter from pustules dried on threads and kept in closed vials for months was known to be effective for variolation Living virus can be isolated from crusts kept at room temperature for over a year; from vesicle or pustule fluid dried on glass slides virus can be recovered after several months. Refrigeration is unnecessary when such specimens are transmitted by post to the diagnostic laboratory The survival of virus on bedclothes of patients has been responsible for transmission of smallpox to laundry workers (Stallybrass, 1931, Cramb, 1951) When diluted in broth and kept in sealed tubes vesicle fluid remains infective for years at 4° C. or −20° C The virus is relatively resistant to 50 per cent glycerol, to phenol and some of the common disinfectants but susceptible to oxidizing agents such as potassium permanganate. The infectivity (for the monkey) of variolous crusts suspended in saline solution is destroyed at 55° C in half an hour (Gordon 1925) Cross-immunity experiments in monkeys show that the viruses of variola, alastrim and vaccinia are immunologically similar. Soluble antigens of variola virus are demonstrable in fluid from smallpox vesicles, in extracts of dried crusts and of infected chorio-allantoic membranes by precipitation or complement-fixation tests using smallpox convalescent serum or rabbit antivaccinial serum These serologic reactions are used in diagnostic tests While the soluble antigens of variola have not been studied as carefully as those of vaccinia, they are practically identical in reactivity with immune sera Hemagglutinin is produced by variola virus, although on the infected chorio-allantois its titer is lower than is the vaccinia hemagglutinin from the same source (North, 1944). No difference between these hemagglutinins has been detected by inhibition tests with immune sera Further reference is made to the immunologic relationships of variola and vaccinia in the section dealing with vaccinia virus

Diagnosis

In the majority of cases of smallpox the diagnosis can readily be made on clinical grounds During the eruptive phase the following points are of importance: the size and the evolution of the lesions within accepted time limits through various stages, the distribution of the eruption over the skin surface, the evidence of progressive eruption with older lesions on the face and the arms and the more recent on the lower limbs but the lesions being homogeneous on any one part The history of a severe febrile pre-eruptive illness

lasting 2 to 4 days is usual, and a definite or possible history of contact with a previous case should be sought for. The clinical diagnosis cannot be made with certainty during the pre-eruptive illness, but such illness in smallpox contacts should be regarded provisionally as variolous. When a diagnosis of smallpox is considered on clinical grounds preventive measures should be instituted without waiting for laboratory tests. The 2 types most likely to be overlooked are the acute hemorrhagic patient who dies before the focal eruption appears and the modified case with a very sparse or atypical eruption in a previously vaccinated individual. The acute hemorrhagic case may be mistakenly diagnosed as acute purpura, typhus, hemorrhagic scarlet fever or acute meningococcal septicemia. The case of variola major modified by previous vaccination, or alastrim in a vaccinated or unvaccinated person, may be confused most commonly with varicella, with pustular acne, with drug eruptions, particularly a pustular sulfonamide rash, or with erythema multiforme and Stevens-Johnson syndrome. Vaccination as a diagnostic procedure may be useful, for less than 10 per cent of smallpox cases can be vaccinated on the first day of the focal rash, and none after the 6th day (Marsden, 1948). If after careful consideration of the history and the clinical picture the diagnosis is still in doubt, the virus laboratory can give valuable assistance.

The laboratory procedures which have been found useful in recent years are directed to: (1) the detection of virus, (a) by direct microscopic examination of material from lesions in the skin and the mucous membranes, or (b) by inoculation on the chorio-allantois of developing chick embryos of material from the blood or skin lesions, (2) the demonstration of specific antigen in focal lesions, and (3) the demonstration of antibody or increase of antibody in the blood during the course of

TABLE 30. LABORATORY TESTS IN DIAGNOSIS OF SMALLPOX

STAGE OF ILLNESS	MATERIAL TO BE SUBMITTED	MICROSCOPIC EXAMINATION OF SMEARS FROM SKIN LESIONS	CULTURE ON CHICK EMBRYO CHORIO-ALLANTOIS	DETECTION OF ANTIGEN BY COMPLEMENT-FIXATION TEST	DETECTION OF ANTIBODY *
Pre-eruptive illness	Blood		±	±	−
Macular and papular stage	Smears from skin lesions	+	+	±	
	Blood				−
Vesicular	Vesicle fluid	+	+	+	
	Smears	+	+	+	
	Blood				±
Pustular	Pustule fluid	±	+	+	
	Smears	+	+	+	
	Blood				±
Crusting stage	Crusts	−	+	+	
	Blood				+
Later	Blood				+
Time required for completion of test		30 min	2-3 days	24 hr	24 hr.

* [illegible]

the illness Table 30 illustrates the material to be sent and the tests likely to give a positive result at various stages of the disease. Details of the method of collecting specimens and the technics used in their examination are to be found in articles by MacCallum (1954) and Kempe (1956). Downie and Macdonald (1953) have recorded the results obtained by the various tests in series of cases of variola major and variola minor.

The presence of elementary bodies in smears from lesions was first used as an aid to diagnosis by Paschen, and the test was found to be of special value by van Rooyen and Illingworth (1944) during an outbreak of smallpox in the Middle East. Much depends on the care with which the preparations are made and the experience of the virologist. Smears on clean slides should be made with material obtained by scraping with the point of a knife or a Hagedorn needle maculopapular lesions or the base of vesicles or pustules Paschen's or Gutstein's staining methods give satisfactory results, but the appearances may be difficult to interpret in preparations from pustules owing to the presence of granular debris However, satisfactory results with such material may be obtained by using Gispen's (1952) modification of Morosow's silver impregnation method (de Jong 1956). The finding of numerous typical elementary bodies in microscopic preparations may enable a presumptive positive report to be sent to the physician within an hour of receipt of the specimen at the laboratory Such a finding should be confirmed by the isolation and the identification of virus on the chorio-allantois or by serologic test for specific antigen if suitable material from the patient is available. A negative finding on microscopic examination is of much less value and should be ignored if the clinical picture favors a diagnosis of smallpox. Preparations from vaccinial lesions give the same microscopic picture as those from variola. Material from cases of varicella or herpes simplex reveals few, if any, elementary bodies, and when present they appear smaller and stain less deeply. The virus particles from the lesions of variola may be demonstrated by the electronmicroscope, but this technic is not practicable for routine work in most laboratories.

The isolation and the identification of virus by use of the chick embryo, first used as a routine diagnostic method by Bohls and Irons (1942), is more easily and more certainly effected than by the inoculation of the rabbit cornea. The lesions which variola virus produces on the chorio-allantois are visible after 48 hours but become more typical after 72 hours The specific nature of the lesions should be confirmed by histologic examination and serologic testing of an extract of the membrane by complement-fixation with rabbit antivaccinial serum. The naked-eye appearances of the lesions will usually enable a diagnosis to be made as between variola and generalized vaccinia occurring during a smallpox outbreak. Where doubt remains the differentiation may be made by inoculation of an extract from the infected chorio-allantois into the skin of a rabbit. Lesions may be produced in this tissue by both viruses, but passage to a fresh rabbit will succeed if the material is vaccinial and will fail if it is variolous. A very small amount of material from smallpox cases will give a positive result on the chorio-allantois Smears on glass slides from macular to pustular stage of the eruption, extracted with saline and used for inoculation will almost invariably produce typical pocks. Extracts from crusts are likewise suitable in late cases which have not been diagnosed early in the disease. During the first few days of the febrile illness virus may be isolated from the blood A positive result was obtained at this stage in all 4 hemorrhagic cases examined by Downie et al (1953), and virus was found during the first few days in the blood of most patients who subsequently died. On the other hand, with the technic used, virus was found in the blood early in the illness of only 4 of 25 patients who recovered Material from varicella patients produces no obvious lesions on the chorio-allantois The fluid from the skin eruption in the rare cases of generalized herpes simplex infections will provoke lesions on the chorio-allantois These can be distinguished as a rule by their smaller size and by histologic examination; extracts of the infected membranes give a negative complement-fixation test with antivaccinial serum

The detection of specific antigen in material from skin lesions is made by the complement-fixation technic using as antibody antivac-

cinial serum prepared in the rabbit. Present procedure is based on that used by Craigie and Wishart (1936a) The test always gives a positive result in smallpox cases if sufficient material is available for examination The fluid from 6 vesicles or pustules or 6 crusts should provide an adequate specimen A positive result may be obtained with less material, even with extracts from a few smears on glass slides, but a negative result from inadequate material is of no diagnostic value Fortunately, virus can usually be recovered by egg inoculation from material which is too small in amount to give a reliable serologic test The test for antigen can be recorded only as positive or negative for variola-vaccinia antigen and will not serve to distinguish the occasional case of generalized vaccinia from smallpox In hemorrhagic cases of smallpox both complement-fixing antigen and virus may be found in the blood during the first few days of illness (MacCallum et al, 1950) So far, antigen has not been detected in the serum of patients who are to recover (Downie et al, 1953).

The test for antibodies in the patient's serum may be of value in the diagnosis of atypical or mild cases who have been overlooked at the beginning of an outbreak or in cases of *variola sine eruptione* In this latter type of infection the test for antibody is the only laboratory procedure likely to be of assistance in establishing the diagnosis The specific antibody may be measured by estimating the highest dilution of the serum which will inhibit vaccinial hemagglutination of selected fowl cells or will fix complement in the presence of specific vaccinial or smallpox antigen Collier et al (1950), who used buffalo vaccinial pulp as a source of hemagglutinin, found that specific antihemagglutinins appeared in the serum of smallpox patients about the 5th or 6th day of illness By the complement-fixation technic antibody is not usually detected until the 8th or 9th day The patient who has been previously vaccinated may show a rise in serum antibody several days earlier than antibody appears in the serum of the unvaccinated patient In previously vaccinated individuals a 4-fold or greater rise in titer of antibody, as demonstrated by the examination of 2 serum samples taken some days apart, would be more useful than the test on a single specimen In unvaccinated persons inhibition of vaccinial hemagglutinin at a serum dilution of 1 80 or higher and complement-fixation in a serum dilution of 1 10 or higher would be regarded as indicative of infection with variola, cowpox or vaccinia virus

Histologic examination of biopsy specimens from skin lesions may serve to distinguish cases of modified variola major or alastrim from cases of varicella (de Jong, 1956) In the varicella vesicle its more superficial situation, the absence of reticular degeneration, the presence of multinucleated epithelial cells and the intranuclear inclusions are characteristic and serve to distinguish it from the smallpox lesion

The tests described above are applicable equally to the diagnosis of variola major and variola minor but do not serve to distinguish between them The recent observations of Dinger (1956) and Helbert (1957) indicate that they may be differentiated by careful study of the virulence for the chick embryo of the virus isolated and comparison with known strains from both forms of smallpox

Treatment

No specific therapy for variola is available The use of sulfonamides and antibiotics is indicated in the treatment and the prevention of bacterial complications It has been suggested that the use of these drugs modifies and diminishes the pustular eruption, but controlled trials have not been reported Convalescent smallpox serum has been used in the treatment of severe cases in doses of 30 to 50 ml but without obvious beneficial result (Illingworth and Oliver, 1944, Easton, 1945) The use of immune gamma globulin in the prophylaxis of smallpox is referred to in the section on control

Epidemiology

The ultimate source of infection in smallpox is the person suffering from the disease The contact may be direct or indirect through utensils, clothing or dust infected by the patient There is no evidence that the patient is infective during the incubation period Infected contacts have been allowed freedom of movement among their fellows during their incubation period without spreading the disease (Dixon, 1948) From the public health

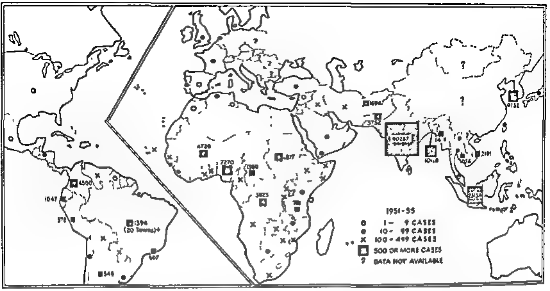

FIG 114 Smallpox, 1951-1955 Annual average of officially reported cases (Modified from Gear, H. S., and Deutschman, Z., 1956, *Disease Control and International Travel*, Geneva, World Health Organ)

point of view the smallpox patient must be regarded as potentially infectious from the onset of illness until the last scabs have dropped off, but epidemiologic observations suggest that most patients become infectious only with the appearance of the focal rash, a view held by Ricketts and Byles (1908) and supported by recent experience By the time the eruption appears there is a large amount of virus in the lower layers of the epidermis, but the impermeable nature of this tissue prevents the virus from reaching the exterior until rupture of vesicles and pustules

early erosion of lesions on the mucous membranes ensures heavy contamination of the saliva and mucous secretions. the virus may be demonstrated in the saliva when the focal rash appears but not usually before. Purpuric cases of smallpox, in whom hemorrhages may occur from mucous membranes soon after onset of illness, may be infectious throughout the febrile illness (Stallybrass, 1931). Therefore, in the early stage of the focal eruption, infection is spread from the mouth and the nose and later from the skin lesions. Because of the resistant nature of the virus, infec-

cotton. MacCallum and McDonald (1957), who have studied the survival of virus in smallpox scabs wrapped in cotton and kept at temperatures from 30° to 40° C. or higher, found that virus did not survive longer than 6 months; but at 20° to 25° C virus could be recovered after 17 months It may be that contacts protected by vaccination and showing no signs of illness may temporarily spread infection A more dangerous source is the vaccinated person whose immunity has waned and whose smallpox infection is so mild that medical help is not sought, or whose infection is atypical and mistaken for varicella The first cases in the 1950-51 outbreak mentioned above and in the Glasgow outbreak of 1950 (Laidlaw and Horne, 1950) illustrate the importance in the spread of smallpox of mild illness in partially immune persons

Unvaccinated persons of all races and ages are susceptible to smallpox The disease may occur at all seasons in the year but is more common in the colder months of winter and spring than in summer. Smallpox is still endemic in large areas of the world but especially in India, some areas of southeast Asia, parts of Africa and some of the countries of South America Other countries are at risk from these reservoirs of infection Movement

demic infections. During and immediately

after World War II there was some spread of smallpox particularly in Africa and Asia (Fabre, 1948), but since then the incidence of the disease has diminished in many countries (Gear and Deutschman, 1956) Figure 114 shows the average annual incidence of officially reported cases in various parts of the World during the years 1951-1955 During this period Europe has suffered little, apart from sporadic outbreaks due to importations from Asia or Africa, and in 1956 no cases were reported in Europe, although information was not available from Albania East Germany, Bulgaria, Poland Rumania or Czechoslovakia (Report 1957) In the U S A the incidence of smallpox was high during the 1920-30 decade, subsequently, there was a remarkable fall in the number of cases recorded (Fig 115) From the year 1945 when there were 346 cases the numbers steadily diminished There were only 4 cases in 1953, and no case of smallpox was recorded in 1954, 1955 and 1956 In certain of the countries of South America (Venezuela, Peru and Chile) the position in recent years has greatly improved coincident with the institution of more general vaccination (Gear and Deutschman, 1956)

Variola major and variola minor seem to be equally infectious But control of the milder disease is more difficult because notification is apt to be incomplete, and missed cases, ambulant while still infectious, tend to prolong an outbreak This is illustrated by the experience of smallpox in Britain in the years 1944-53 Many small outbreaks of variola major resulted from importations into England, the largest number in any one outbreak being that in 1953 (30 cases with 8 deaths) However, the alastrim outbreak in 1951-52 extended over 4½ months, there were over 140 cases but no deaths

Control Measures

Measures for the control of smallpox recommended by the American Public Health Association (1955) have been approved in principle by the U S Public Health Service and the Armed Forces as well as by departments of health of foreign governments These recommendations are made under 4 headings and are summarized below

A Preventive Measures

1 Vaccination at about 3 months of age with revaccination at school entry and on exposure to high risk of infection

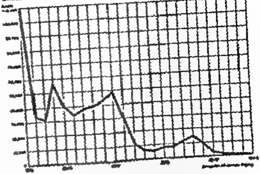

Fig 115 Cases of smallpox reported in the United States, 1921-1944 (Dublin, L I, 1945, The conquest of smallpox, Statistical Bulletin Metropolitan Life Insurance Company *29*, 8)

2 Measures to ensure available supply of potent lymph kept below freezing point to maintain potency

3 Vaccination by multiple pressure on small area of skin over deltoid preferred, no dressing need be applied Children with eczema should not be vaccinated or come into contact with recently vaccinated persons After revaccination examination should be made at 3rd and 9th days to determine whether reaction is of primary, vaccinoid or early (immediate) type Early reaction difficult to interpret If in doubt revaccinate If no reaction always revaccinate

B Control of infected individuals, contacts and environment

1 and 2 All cases to be reported to Health Authority and isolated in hospital until all crusts have disappeared

3 and 4 All oral and nasal discharges and articles associated with patients to be disinfected by burning, high pressure steam or boiling

5 and 6 All contacts at home, place of work or elsewhere to be vaccinated or revaccinated with potent lymph and kept under surveillance for 16 days from time of last contact Any rise of temperature during surveillance calls for prompt isolation until smallpox can be excluded

7 The immediately prior case should be sought assiduously Adult chickenpox or patients with hemorrhagic or pustular lesions of the skin need careful review for errors in diagnosis

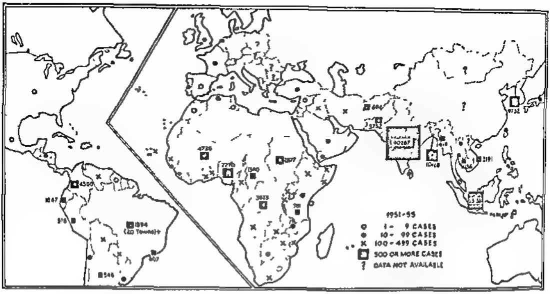

FIG 114 Smallpox, 1951-1955. Annual average of officially reported cases (Modified from Gear, H. S., and Deutschman, Z., 1956, Disease Control and International Travel, Geneva, World Health Organ)

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shire, England, have been traced to imported cotton. MacCallum and McDonald (1957), who have studied the survival of virus in smallpox scabs wrapped in cotton and kept at temperatures from 30° to 40° C or higher, found that virus did not survive longer than 6 months, but at 20° to 25° C virus could be recovered after 17 months. It may be that contacts protected by vaccination and showing no signs of illness may temporarily spread infection. A more dangerous source is the vaccinated person whose immunity has waned and whose smallpox infection is so mild that medical help is not sought, or whose infection is atypical and mistaken for varicella The first cases in the 1950-51 outbreak mentioned above and in the Glasgow outbreak of 1950 (Laidlaw and Horne, 1950) illustrate the importance in the spread of smallpox of mild illness in partially immune persons

Unvaccinated persons of all races and ages are susceptible to smallpox. The disease may occur at all seasons in the year but is more common in the colder months of winter and spring than in summer. Smallpox is still endemic in large areas of the world but especially in India, some areas of southeast Asia, parts of Africa and some of the countries of South America Other countries are at risk from these reservoirs of infection. Movement of populations, the disruption of public health control measures and other factors associated with war tend to favor the spread of endemic infections During and immediately

ice personnel were not always reliable, certainly less reliable than the evidence of vaccination scars (Easton, 1945; Waldo, 1955). The occurrence of smallpox in soldiers recorded as having been successfully vaccinated gave rise to some doubt as to the ability of the vaccine then in use to protect against the virulent strains of variola virus encountered. However, the fatal cases of smallpox in outbreaks among troops in the Middle East and the Far East where data were recorded were usually devoid of vaccination scars.

It should be emphasized that faulty vaccine or faulty technic may result in failure to produce a reaction on human skin. Such absence of reaction should not be regarded as evidence of immunity but as an indication of the need for revaccination with potent lymph. Easton (1945) records of one man who died of confluent smallpox that vaccination had been attempted at birth, again in 1941 and 10 times in 1943 without a take, thus emphasizing the danger of accepting even repeated unsuccessful vaccination as evidence of insusceptibility to smallpox.

Vaccination is a relatively safe and very effective immunizing procedure. The serious complications which may occasionally follow are generalized vaccinia and postvaccinial encephalitis. Serious infection with pyogenic bacteria and postvaccinial tetanus are rarely encountered today. Generalized vaccinia occurred in 45 persons following the mass vaccination of 5,000,000 persons in New York during 2 months in 1947 (Greenberg, 1948). Of the 45 cases of generalized vaccinia, 38 had a pre-existing dermatosis, and 28 of them had not been vaccinated but were accidentally infected through contact with vaccinated persons. Of the last group 2 babies under 1 year of age died. Among the 5,000,000 vaccinated, 44 cases and 4 deaths were attributed to postvaccinial encephalitis. In the 4 patients who died histopathologic examination did not show changes typical of postvaccinial encephalitis. The incidence of this complication varies at different times and in different countries (Stuart, 1947-48). In Scotland following smallpox outbreaks in 1942 the incidence of postvaccinial encephalitis in Glasgow was 1 per 70,000 vaccinations, and in Edinburgh and district 1 per 21,000 vaccinations. In Holland the incidence has been unusually high, but the cause of this is unknown. It is believed to be uncommon in the first year of life, but Herrlich (1954) records an incidence of 1 in 17,400 vaccinations under 1 year of age in Bavaria for the years 1945-1953.

Immunity following successful vaccination develops rather more quickly than that following smallpox infection by the usual route, and it has been maintained in the past that successful vaccination within 3 days after exposure would protect previously unvaccinated contacts against smallpox. While this may have some general validity there are exceptional cases, for smallpox has been recorded in contacts successfully vaccinated within 1 day after exposure. Of 1,083 cases of variola minor, who had been vaccinated successfully after exposure to infection, the focal rash appeared in 89 patients on the 12th day, in 17 patients on the 13th day, in 5 patients on the 14th day, and in 1 patient on the 15th day after the vaccination (Marsden, 1948). Such observations serve to stress the need to avoid delay in the vaccination of smallpox contacts. The time of appearance and duration of immunity following vaccination is variable, and whether or not smallpox develops in a vaccinated person depends not only on the nature of the person's immunity response to the vaccination but also on the intensity of subsequent exposure to infection. Revaccination at frequent intervals is advisable for persons exposed to high risk of infection, such as troops serving overseas in endemic areas. The need for vaccination and revaccination of the staffs of hospitals is forcefully emphasized by 2 outbreaks in British hospitals which had admitted missed cases of variola. Of the total of 48 cases of smallpox in those outbreaks 23 were members of hospital staff, and 11 of these died (Laidlaw and Horne, 1950; Cramb, 1951).

The standard smallpox vaccine is glycerinated calf lymph or, in England, glycerinated sheep lymph. These glycerinated lymphs must be stored by the manufacturer at $-10°$ C. After distribution, vaccine may be used in the U.S.A. up to 3 months if stored below 10° C. More stable preparations are needed, especially in the tropics where refrigeration during storage and transportation is difficult. Dried buffalo lymph prepared by Otten et al (1950) has been used successfully on a wide

C. Epidemic measures.

1 and 2 Hospital isolation of cases and suspects until no longer infectious. Careful listing of all contacts, vaccination and surveillance for 16 days.

3 Immediate publicity by available methods giving frank statement of situation and urging individuals in area to be vaccinated. Provision of potent vaccine and arrangements for vaccination

4. Mass immunization of whole population of community or larger area is an emergency measure to be used when an outbreak has given evidence of material spread.

D. International measures

1 Telegraphic notification of W.H.O. and adjacent countries of existence of epidemic of smallpox

2 Measures applicable to ships, aircraft and land transport arriving from smallpox areas are specified in International Sanitary Regulations (W.H O Techn Rept Ser. No. 41 Geneva, 1951).

3 Evidence of a previous attack of smallpox or of recent vaccination or revaccination (within a period of 3 years) is a widely enforced requirement for entry to or departure from a country

The smallpox vaccine in common use is prepared from the vaccinial lesions on the skin of inoculated calves or sheep, and contains infective virus Methods to be used in its preparation, preservation and storage until used for vaccinating human beings against smallpox are carefully controlled Regulations in force in the United States regarding these matters have been set forth by the National Institutes of Health (1946) For the United Kingdom they are embodied in the Therapeutics Substances Regulations, 1952, No. 1937, amended by Regulations 1957, No. 550. (London Her Majesty's Stationery Office.)

The reactions resulting from the inoculation of smallpox vaccine are of 3 general types: namely, primary, vaccinoid or accelerated and early or immediate (previously called immune) These 3 types of reaction at different stages of their development were well illustrated in the colored plate (page 424) in the 2nd edition of this book (1952) A primary reaction is that which normally follows in a person who has not previously had experience of smallpox, cowpox or vaccinia virus. Until the 4th day following vaccination there is no reaction. Then a small papule appears which rapidly becomes a vesicle. This enlarges in size, and there develops around it a zone of erythema of variable width. The center of the vesicle usually becomes depressed, and by the 8th or 9th day the contents become turbid, and there may be associated axillary adenitis with some degree of fever The reaction reaches its maximum extent usually between the 8th and the 10th days, after which the pustule dries up, and a scab forms which may take a week to separate This reaction is usually followed by a variable degree of immunity to vaccinia and variola beginning about the 10th day and lasting for months or years. Babies born of recently vaccinated mothers possess antibodies and may show a poor response to vaccination during the first few weeks of life.

The vaccinoid or accelerated reaction following inoculation with a potent lymph is a modified reaction given by a person who has some residual immunity from a previous attack of smallpox or vaccination The maximum intensity is reached between the 3rd and the 7th days following vaccination Although the lesion is smaller than the primary reaction, vesiculation always occurs This type of response increases the waning immunity of previous vaccination

The early or immediate reaction is an indication of sensitivity to the virus and may be given by persons who are either susceptible or immune to smallpox. The reaction is most marked on the 2nd or 3rd day and diminishes thereafter. The local lesion is usually papular but may vesiculate. This response has been called an immediate reaction of immunity, but it is neither immediate nor necessarily a reaction of immunity It may occur in those whose immunity has waned and may be elicited by inoculation of dead virus (Hooker, 1929, Broom, 1947, Benenson, et al, 1952) When a potent vaccine is used the early reaction may merge into the vaccinoid reaction But the early reaction by itself cannot be regarded as a successful result and cannot be guaranteed to induce or increase the person's resistance to smallpox The interpretation of an early reaction as a successful vaccination indicating a state of immunity may have been, in part, responsible for reports of smallpox, sometimes fatal, in successfully revaccinated service personnel Vaccination records of serv-

cows the true cowpox lesion appears on the teats or on the contiguous part of the udder as small red pimples which increase in size and become vesicular, the vesicles which surmount a firm deep-seated inflamed base quickly show a depressed center and tend to be ruptured early during the operation of milking The ruptured surface becomes covered with scabs which take some time to separate, and several weeks may elapse before the lesions heal As a rule, the animals do not suffer serious general upset The disease tends to spread quickly through a herd, infection being conveyed from one animal to another in the process of milking In those attending affected animals one or more lesions may appear on the hands—the thumb, the first interdigital cleft and the forefinger being especially liable to attack, but scratches or abrasions of the skin may determine localization of the lesions elsewhere on the hands, the forearms or the face The lesions in their development resemble those of primary vaccination Local edema is often pronounced, there are lymphangitis and lymphadenitis, and there may be some degree of fever for several days, especially in those who have never been vaccinated In such persons 4 or 5 weeks may elapse before the scabs finally separate Second attacks may occur after an interval of years but in these, as in persons who have been previously vaccinated, there may be no fever or constitutional upset

Pathologic Picture and Experimental Infection

The pathologic changes produced and the natural and experimental host range are similar to those of vaccinia virus Histologically, the lesions in the skin of those infected with cowpox virus from cattle show great epidermal thickening, rather less rapid cell necrosis than in vaccinia and the presence of numerous large homogeneous strongly acidophilic cytoplasmic inclusions in the lower layers of the affected epidermis Leukocytic infiltration, congestion and hemorrhage are to be found in the dermis immediately underlying the affected epidermis The virus can readily be maintained by passage in the skin or the testicle of the rabbit, by intradermal inoculation of guinea pigs, mice or monkeys, or on the chorio-allantois of the chick embryo In all these tissues cowpox virus differs from dermal strains of vaccinia in that there is less rapid necrosis of epithelial cells which exhibit the large inclusions above mentioned, there is a greater tendency to invade mesodermal tissue, and by involvement of capillary endothelium the lesions in skin and chorio-allantois have a characteristic hemorrhagic appearance (Downie, 1939a; Dekking, 1950; Verlinde, 1951; Herrlich and Mayr, 1955) In the rabbit cornea lesions are less extensive than those produced by vaccinia There is less epithelial necrosis, and the cytoplasmic inclusions are more strongly acidophilic and much larger than classic Guarnieri bodies (Berger, 1956) In the rabbit fatal generalized infection may result from intravenous inoculation of virus, and inoculation of large doses on the chorio-allantois may lead to fatal infection of the chick embryo A variant has been isolated from cowpox strains grown on the chorio-allantois of chick embryos (Downie and Haddock, 1952, van Tongeren, 1952) This variant resembles dermal strains of vaccinia in that the lesions are associated with rapid necrosis of infected epithelium, a marked polynuclear leukocytic reaction and absence of hemorrhage, but the lesions show the large cytoplasmic inclusions characteristic of the normal cowpox strains

Etiology

The virus of cowpox is identical in size and appearance with that of vaccinia when examined by the electronmicroscope, and in so far as it has been examined it is similar in resistance to heat and chemical agents The antigens of cowpox virus have not been studied so intensively as those of vaccinia but have the same serologic reactivity The hemagglutinin is not obtained in such high titer from infected tissue but acts on the same range of fowl red cells The LS antigen (Smadel, 1952) is similar, although by cross complement-fixation tests with immune sera there is a slight difference in the soluble antigens of the 2 viruses Precipitation studies by the agar gel diffusion technic suggest that there is a quantitative difference in minor antigenic components (Gispen, 1955) Cross-absorption of anticowpox and antivaccinial sera prepared in rabbits or fowls, using living virus suspensions to absorb the sera, confirm that although there is a very large antigenic overlap there are minor antigenic differences between the strains

scale in Indonesia since 1946. Collier (1955) found that a partially purified elementary body suspension prepared from sheep pulp, suspended in 5 per cent peptone, freeze-dried and sealed in vacuo kept its potency well for some months at 37° C and 45° C. This type of vaccine, even after storage for 2 years at 37° and at 45° C., showed practically no loss of titer on the chorio-allantois or rabbit skin and gave 100 per cent successful primary takes on previously unvaccinated persons (Cockburn et al, 1957). One disadvantage of lymphs prepared in animals is the difficulty of obtaining a product completely free from bacterial contaminants. To obtain this desirable objective 2 other sources of virus have been used for the preparation of vaccine—vaccinia virus propagated in the chorio-allantois of chick embryos and in tissue culture Glycerolated vaccine prepared from virus grown on the chorio-allantois has been used in certain parts of the U.S.A. for some years After 240 continuous egg passages, however, the strain used for vaccine preparation gave milder lesions in vaccinated children and a slightly greater susceptibility to revaccination after 14 months than did calf-lymph vaccine (Buddingh, 1943) *Subsequently, satisfactory* vaccine was prepared by the egg method using as inoculum calf lymph to which penicillin and streptomycin had been added (Buddingh and Randall, 1951) Similar seed virus has been used for the preparation of egg vaccine by the Texas State Department of Health, and this vaccine has given satisfactory results in the field for a number of years (Cook et al, 1953) A method of preparing stable bacteria-free lyophilized vaccine from the chick chorio-allantois has been elaborated by Jackson et al (1956) In recent years vaccinia virus grown in tissue cultures of bovine embryo skin has been used in the preparation of smallpox vaccine (Wesslén, 1955). This method also provides a bacteriologically sterile vaccine which can be produced very economically. It has not yet been demonstrated that either of these vaccines minimizes the risk of postinfectious encephalitis, their other advantages may yet overcome the conservatism which has prevented the wider adoption of potent bacteria-free egg vaccine.

It has been indicated above that vaccination of smallpox contacts may be effected too late to ensure protection from the disease. In these cases it seems that passive protection might be achieved by the administration of immune gamma globulin early in the incubation period, as in measles. Preliminary trials of this procedure have been made by Kempe et al (1956), using gamma globulin prepared from the blood of recently vaccinated persons Among the 56 contacts in the test group who were vaccinated and given immune gamma globulin there were 2 cases of smallpox—1 fatal and 1 modified; while in the control group of 75 contacts, who were comparable in age and vaccination history with the test group and were vaccinated only, there were 8 cases of smallpox and 3 deaths Although the numbers in the 2 groups were small, the results seem to suggest that this method of prophylaxis is worthy of further trial in epidemic areas

COWPOX

History

Cowpox was first brought to the notice of the medical profession generally by Jenner's publication on this disease in 1798, but there is no doubt that it had long existed in cattle as an endemic disease which was occasionally transmitted to milkers Cowpox still occurs in Europe, but it is not a serious disease in cows and attracts medical attention when the infection is contracted by those engaged in milking or otherwise caring for the affected animals

Clinical Picture

The disease in cattle was described in great detail by Ceely (1840) who, like Jenner, was careful to distinguish true cowpox from other eruptions seen on the teats of cows It seems clear from more recent literature that, even at the present time, this distinction is not always made The so-called natural cowpox said to be prevalent in America and causing milkers' nodes in man (Hester et al, 1941; Nomland and McKee, 1952), is etiologically unrelated to Jenner's true cowpox There is no cross-immunity between the virus responsible for milkers' nodes and the virus of vaccinia (Becker, 1940, Berger, 1955), whereas cowpox virus and vaccinia are immunologically almost identical Moreover, the clinical and histologic findings in milkers' nodes are quite different from those of cowpox in man. In

us is that of herpes simplex Eczema vaccinatum may be a local infection resulting from implantation of virus on eczematous skin, but the simultaneous appearance of the eruption, the occurrence of lesions on healthy skin and the fact that the dermatitis may be quiescent at the time the vaccinial infection is acquired would suggest that blood-borne virus localizes in the abnormal areas of skin or that there is an extremely rapid spread, possibly by skin lymphatics, from the original site of virus implantation

Generalized vaccinia occurring in persons with previously healthy skin is a much rarer condition with onset between the 9th and the 14th days after vaccination The condition may resemble smallpox, although the eruption does not usually have such a marked centrifugal distribution The lesions may appear in crops over 2 or 3 days Usually, the condition is not fatal, and rapid recovery is the rule This type of generalized infection seems to be associated with delayed antibody response to vaccination

Fortunately, progressive vaccinia is very rare Following vaccination there may be a normal primary take, but the lesion does not heal Instead, it extends slowly with necrosis of tissue, and fresh vesicles appear in the neighborhood of the vaccination site or in other regions of the body The mucosa of the mouth, the pharynx and the larynx may become involved, and the child slowly loses weight but may survive for 3 or 4 months after vaccination (Bigler and Slotkowski, 1951), the successive appearance of fresh lesions on various skin areas indicates a continuing viremia, and in the case reported by Keidan et al (1953) virus was recovered from the blood on 4 occasions from the 21st to the 39th day after vaccination There may be a leukopenia affecting especially lymphocytes Histologic study of postmortem material may show a complete absence of reactive inflammation around the skin lesions, areas of focal necrosis found in lungs, liver, adrenal and kidney in the case reported by Hall et al (1953) showed a similar absence of cellular reaction. In all cases reported in which examination for neutralizing antibody has been made none has been found, in a few, though not in all, there has been agammaglobulinemia (Kempe et al, 1956)

Pathology

The lesion produced in the skin by vaccinia virus, whether in man or in animals, is histologically similar to that seen in smallpox The epithelium undergoes the same kind of changes, and when in experimental animals the skin lesions result from generalization by the blood stream, evidence of epithelial damage may be preceded by changes around the small vessels in the dermis, these show dilatation and swelling of endothelium, and there may be infiltration of mononuclear cells around them In experimental animals the nature and the extent of the lesions varies with the strain of virus, the size of the infecting dose and the route of inoculation In generalized infections lesions of the skin of the eyelids, the mucocutaneous junctions about the lips, the nares and the anus are common Areas of focal necrosis, sometimes surrounded by a zone of congestion or hemorrhage, may be encountered in liver, lungs, kidneys, ovaries and testis Lesions produced on the chick embryo chorio-allantois may be 2 to 3 mm in diameter after 3 days The infected cells in the greatly thickened ectoderm tend to become rounded off and show acidophilic inclusion material occupying a large part of the cytoplasm Inclusion bodies are found less commonly in the cells of the edematous mesoderm and in the hypertrophied endoderm If the inoculum is large, lesions may appear on the skin and the internal organs of those embryos which survive for 4 or 5 days

Experimental Infection, Host Range

A wide range of animals is susceptible to infection with vaccinia virus Apart from the infections in man which have been considered above, calves, sheep and rabbits have been regularly employed for the propagation of virus in the production of vaccine lymph for smallpox vaccination Other laboratory animals, such as monkeys, rats, mice, guinea pigs and hamsters, may also be infected, although they are less susceptible than the rabbit to strains commonly used The chick embryo may be infected by inoculation of virus on the chorio-allantois, into the amniotic cavity or into the yolk sac, although the lethal dose for the embryo by the first route may be only a hundredth of that by the other two routes (Cabasso and Moore, 1957)

Strains of vaccinia virus used for the production of smallpox vaccine in various labora-

of cowpox and vaccinia viruses so far examined (Downie, 1939b; Downie and McCarthy, 1950).

Diagnosis

Clinically, cowpox in farm workers may resemble 2 other virus infections occasionally acquired through contact with stock animals, namely, milkers' nodes from cows and contagious pustular dermatitis from sheep. These infections are less vesicular in character and are usually less severe in man than that occasioned by cowpox virus, in neither can transmission to laboratory animals be readily effected. The clinical diagnosis of human cowpox infections may be confirmed by the laboratory technics used in the diagnosis of smallpox The isolation of virus from the patient by inoculation of vesicle fluid on the chorioallantois and the appearance and the histology of the focal lesions in this tissue or in the skin of the rabbit will serve to distinguish infections caused by cowpox virus from those occasionally contracted from cows which have been accidentally infected with vaccinia strains (see below).

Epidemiology

Infection in man is acquired from infected cows and rarely spreads from person to person In a herd of cows an outbreak can often be traced to an animal recently brought to the farm from outside It has been suggested that infection in cows results from contact with a recently vaccinated person (Hamburger, 1948) That cows may be so infected and transmit the infection again to man is indicated by the findings of Dekking (1955), of 36 strains isolated from outbreaks in Holland 8 had the characters of laboratory vaccinia strains, and the remainder were typical cowpox strains All strains isolated from outbreaks in England during the last 20 years have been typical cowpox virus

Treatment and Control

There is no specific treatment The disease in cows does not appear to be sufficiently serious or widespread to warrant prophylactic vaccination.

VACCINIA

Introduction

The term vaccinia is used here to indicate the infection which is caused by the virus propagated in laboratories and used for prophylactic vaccination against smallpox. As indicated above, the history and the origin of many of the strains used for this purpose are obscure. While certain strains are alleged to have been derived from smallpox, it seems to the writer that the properties and the characters of most strains now in use suggest that they have been derived from cowpox virus, i e, the virus responsible for the pox disease of cows Strains of vaccinia virus by repeated passage in different animals or tissues may acquire characters which serve to distinguish them from vaccine lymph strains, but it is those strains which have been passed repeatedly by dermal inoculation on cows, sheep or rabbits that are considered below, as such strains have been studied most intensively and are best characterized.

Clinical Picture

The infection commonly produced by inoculation of vaccinia virus on human skin has been described above. Occasionally, the illness which follows vaccination is much more severe Secondary lesions may arise elsewhere on the skin through transfer of infection, usually by the fingers, from the initial lesion at the site of inoculation. But more generalized infection is sometimes seen, and this may present 1 of 3 distinct clinical pictures: eczema vaccinatum, true generalized vaccinia and progressive vaccinia (vaccinia gangrenosa)

Eczema vaccinatum may occur as a complication of vaccination in children, or occasionally in adults, who suffer from chronic dermatitis. But in some instances the infection is acquired by unvaccinated children through contact with a recently vaccinated person Groups of cases have occurred in dermatologic wards in hospital (Sommerville et al, 1951). In these patients there may be a febrile illness before the appearance of the eruption. Vesicles appear rapidly and are usually umbilicated. The lesions occur for the most part on areas of skin subject to eczema, although it may not extend to all such areas, and vesicles may appear in previously healthy skin. There is usually adenitis of regional lymph nodes, and high fever is common. The condition may be clinically indistinguishable from eczema herpeticum in which the causal

readily in stained preparations with the ordinary light microscope or in unstained preparations by darkground illumination or by phase-contrast microscopy, as small spherical bodies of approximately 0 2 μ in size. Estimates of size obtained by ultrafiltration through gradocol membranes by Andrewes and Elford (1932) were rather less (0 125 to 0 175 μ) The figures calculated from ultracentrifugation data have varied in the range from 125 to 252 mμ. Measurement of particles in electronmicrographs gives values about 230 to 300 mμ The density of elementary bodies has been determined by the sedimentation rate of the particles in fluids of different specific gravity Because of different osmotic effects exerted by the suspending fluids used—urea, glycerol and sucrose—the density of the virus has been found to vary between that of bacteria 1 10 and that of proteins 1 33

Electronmicroscopy has contributed much to our knowledge of the size and the structure of vaccinia virus The observations made by Green et al (1942) on dried films of elementary bodies indicated that the virus under these conditions was brick-shaped and contained a central area more opaque to the electron beam Short treatment with 0 02 N NaOH increased the prominence of the central area Extraction with 0 1 N NaOH laked the particles, leaving empty ghosts Treatment of elementary bodies with crystalline pepsin dissolved three quarters of the virus substance but left the central body, 90 mμ in diameter, apparently unaltered (Dawson and McFarlane, 1948), all the phosphorus and the desoxyribonucleic acid remained in the residue Incubation with desoxyribonuclease liberated the nucleic acid in soluble form with some loss of density of the central body In preparations from infected chorio-allantois or rabbit cornea electronmicrographs revealed a greater variation in size of elementary bodies (240 to 380 mμ × 170 to 270 mμ) than that seen in preparations from purified suspensions (Peters and Nasemann, 1952) The results of enzymatic digestion varied with the method of fixation, but after Chabaud fixation and treatment with pepsin many of the elementary bodies showed well-differentiated outer membrane and central body Treatment with desoxyribonuclease did not remove the inner body but so altered it that further treatment with pepsin left membranes empty (Stoeckinius and Peters, 1955). The inner body appears to be composed of desoxyribonucleoprotein, and the appearances seen after various enzymatic treatments were similar to those observed in preparation of *E. coli* and *Neisseria sicca* (Peters and Stoeckinius, 1954) The electronmicroscopic examination of thin sections of vaccinia-infected tissue have served to show the relation of intra-cellular virus to other cell constituents (Wyckoff, 1951), although with this technic the problems of artefact production are considerable. The virus particles all appear oval or round but vary in size, density and inner structure (Gaylord and Melnick, 1953). Many particles had incomplete membranes and before release from the cell often showed a double membrane (Morgan et al, 1954) In the absence of sequential studies, correlated with biologic observation, the interpretation of these findings in relation to a developmental cycle of the virus must remain conjectural

The virus of vaccinia has a complex chemical structure Protein, nucleic acid (DNA), phospholipid, neutral fat and carbohydrate have been found in purified elementary body suspensions in amounts similar to those of bacteria and mammalian cells Chemical analysis of purified elementary bodies showed the following percentage composition in dry weight carbon 33 7, nitrogen 15 3, phosphorus 0 57, copper 0 05, total lipids 5 7 (consisting of cholesterol 1 4, phospholipid 2 2 and neutral fat 2 2), reducing sugars 2 8, thymonucleic acid 5 6 (Smadel, 1952) Of these cholesterol could be removed by ether extraction without altering the infectivity of the preparations Practically all the phosphorus and the reducing sugars are contained in the thymonucleic acid fraction Vaccinia elementary bodies also contain biotin and flavin Phosphatase, catalase and lipase activity have been found in suspensions of elementary bodies, but it is uncertain whether these enzymes are integral constituents of the virus and not adsorbed from the host cells Some information as to the probable distribution of genetic material in the vaccinia elementary body is available from the observations of Lea and Salaman (1942) As a result of studies on the inactivation of virus by exposure to alpha rays, x-rays and gamma

tories or institutes vary in their effects on animals The method of routine propagation of these strains may determine the greater tendency of some to induce fatal encephalitis on injection into rabbits (Herzberg, 1955) The strains examined by Herrlich and Mayr (1954) showed fairly constant differences in the nature of the lesions produced in the rabbit skin and on the chorio-allantois, and similar differences have been noted by Fenner (1958) The strains that have been repeatedly passed by scarification on the skin of calves or rabbits—dermal strains—tend to be less virulent for animals than those propagated by repeated passage in the rabbit testis—testicular strains—or in the rabbit brain—*neurovaccinia* The lesions produced by dermal strains in the rabbit skin and on the chorio-allantois tend to be less hemorrhagic than those produced by testicular virus or neurovaccinia These latter strains have a greater ability to grow in mesodermal tissues Strains with high virulence for mice may arise similarly by repeated intracerebral passage in these animals or by frequent intraperitoneal passage in mice with ascites tumors (Cassel, 1957) As previously noted, repeated propagation of vaccinia strains on the chorio-allantois or in chick embryo tissue culture tends to lessen the virulence of vaccinia virus for the rabbit and for man In tissue cultures using HeLa cells cytopathogenic effects may be visible within 48 hours (Ryden and Randall, 1957) It is apparent that the results of experimental infection in different animal species will be determined, among other things, by the history and the properties of the particular strain of virus under study A more detailed account of the effects of vaccinia virus strains on experimental animals has been given by van Rooyen and Rhodes (1948).

Etiology

The virus of vaccinia was probably first seen and depicted by Buist in 1887 (Gordon 1937). This publication was overlooked, and elementary bodies were described by Paschen in 1906 who found them regularly in material from vaccine pustules and in the lesions of smallpox and observed that they were agglutinated by immune sera (Paschen, 1924). The association of infectivity with the elementary bodies was established by centrifugation experiments on Berkefeld filtrates of rabbit dermal pulp extracts (Eagles and Ledingham, 1932) and by studies with collodion membrane filters of graded porosity (Elford and Andrewes, 1932). Perhaps the final proof that the elementary bodies of vaccinia are the virus came from experiments designed to show that lesions could be produced by single elementary bodies (Parker and Rivers, 1936a; Smadel, Rivers and Pickels, 1939). The ratio of virus particles to infective units in such experiments obviously depends on the virulence and the viability of the particles and the susceptibility of the tissue used to determine infectivity, but the fact that the ratio approached unity is also good evidence for believing that the elementary body is the virus. The ratios of particle count to infective units of vaccinia virus have been reinvestigated recently by Overmann and Tamm (1956) and Dumbell et al. (1957), using improved technics of counting virus particles by the electronmicroscope (see Chap. 2).

Vaccinia virus was propagated in laboratory animals for the production of smallpox vaccine many years before its nature was established It was also the first virus to be prepared in a relatively pure state in quantities which permitted detailed examination of its physical, chemical and antigenic structure The preparation of purified suspensions involves the separation of virus from the tissue cells in which it has multiplied. The technics commonly used are based on that of Craigie (1932) who improved on the methods of earlier workers The starting material is the infected epidermis of rabbits that have been inoculated 3 or 4 days before by rubbing into the skin a concentrated suspension of rabbit adapted virus Suspensions of virus may be obtained in the same way from the skin of the sheep or the calf The infected chorio-allantois may also serve for the preparation of virus suspensions by a similar technic, although it is more difficult to free such suspensions from tissue particles (Smadel and Wall, 1937).

A comprehensive account of the properties of vaccinia virus is given in the 2nd edition of this book by Smadel (1952), who with Rivers and their colleagues, have contributed much to our knowledge of the elementary body of vaccinia virus. The virus can be seen

60° C., whereas the S antigen is stable on heating to 90° C. or even higher. This soluble antigen can be concentrated by iso-electric precipitation (Craigie and Wishart, 1936b) and when purified has been found to possess the properties of a protein molecule with molecular weight 240,000 (Smadel and Shedlovsky, 1942). It appears that this protein has 2 antigenic groupings which elicit distinct antibodies. The L and S parts of the antigen can be degraded independently by appropriate treatment (Smadel, 1952). Mayr et al. (1955) in their studies of soluble antigen from various pox viruses could find no evidence of a labile fraction in purified preparations, but it is doubtful if their serologic technics were suitable for the demonstration of the labile moiety of the antigen.

The nucleoprotein antigen is also a serologically specific component of the vaccinia virus (Smadel et al., 1942). It is obtained by extraction of elementary bodies with dilute alkali and constitutes at least half the substance of the virus. It contains 6 per cent thymonucleic acid and precipitates and fixes complement with antivaccinial serum. Injection of noninfectious alkaline extracts of elementary bodies elicits precipitins in rabbits, but such animals are not immune to infection, nor do their sera contain neutralizing antibody.

The LS and NP antigens make up a considerable proportion of the surface of elementary bodies, and these are agglutinated by LS and NP antibodies. After absorption of hyperimmune sera with LS and NP antigen so that the serum no longer precipitates with these antigens, elementary bodies are still agglutinated, although to a lower titer than with unabsorbed sera (Smadel and Shedlovsky, 1942). This residual agglutinin has been designated X agglutinin. Its relation to other antibodies in antivaccinial serum remains undetermined. Vaccinial hemagglutinin (Nagler, 1942) is active against the red cells of only some fowls. The hemagglutinin can be freed from the virus particle by centrifugation, and virus preparations of undiminished infectivity can be obtained by absorbing out the hemagglutinin with suitable red cells (Burnet and Stone, 1946). The hemagglutinin was found by filtration experiments to be a relatively large particle, i.e., 65 mμ (Chu, 1948), although Gillen et al. (1950) thought that there were 2 components—a larger heat stable and a smaller heat labile fraction. Briody (1951) has suggested on the basis of results of treatment with trypsin and ethyl alcohol that the hemagglutinin is polydisperse. It is probably a lipoprotein with phospholipid as the lipid component (Burnet and Stone, 1946). Antibodies to hemagglutinin appear in the serum after vaccinial infection of man or animals, but the antibody is not related to LS or neutralizing antibody. The hemagglutinin of vaccinia is very similar to that produced by variola virus on the chorio-allantois (North, 1944). The antibodies corresponding to LS, NP and hemagglutinating antigens are apparently distinct from the neutralizing antibody present in immune sera. Injection of these separate antigens do not induce immunity or the formation of neutralizing antibody in rabbits, absorption of immune sera by these antigens fails to remove neutralizing antibody. However, absorption of immune sera with appropriate amounts of active elementary bodies removes not only agglutinins and precipitins (LS and NP antibodies) but also neutralizing antibodies (Salaman, 1937). It seems that the antigen associated with the living particle which determines the production of immunity and neutralizing antibody after infection is relatively labile, but its nature is unknown.

The immunologic relationship of cowpox and smallpox was established by Jenner when he showed that individuals recovered from cowpox infection could not be variolated successfully. His observations were confirmed repeatedly by other physicians within a few years of the publication of his findings. Similarly, the immunity induced in man by smallpox against vaccinia has been established by the fact that, after the appearance of the eruption of smallpox, vaccination fails to take (Ricketts and Byles, 1908, Marsden, 1948). The serum of recently vaccinated individuals neutralizes the virus of smallpox when tests are made on the chick embryo chorio-allantois (Buddingh, 1943), and convalescent serum from alastrim patients neutralizes the viruses of vaccinia, cowpox and variola when tested in the same way (McCarthy and Downie 1953). The soluble antigens from tissues infected with variola, vaccinia, cow-

rays, they concluded that vaccinia is a complex of genes and genetically inert material, and that the genes are either dispersed through the particle or confined to a nucleus of a diameter at least half that of the particle The internal structure of the virus as revealed by electronmicroscopy and the amount of nucleoprotein determined by chemical analysis are compatible with the conclusions of Lea and Salaman (1942).

The virus of vaccinia is relatively stable. It may be kept almost indefinitely when stored at $-70°$ C in suitable suspending fluid and will remain active in sealed ampules at ordinary refrigerator temperatures for many months. The virus withstands drying, and when freeze-dried and kept in an atmosphere of nitrogen, preparations maintain their activity for years In fluid suspension vaccinia is destroyed by heat at 60° C for 10 minutes but in dry form withstands 100° C. for 10 minutes Ultraviolet light, alpha rays, x-rays and gamma rays all have a rapidly lethal effect, and the virus is sensitive to the photodynamic action of various dyes in the presence of light (van Rooyen and Rhodes, 1948) The virus in vaccine lymph containing 50 per cent glycerol survives for months at 4° C and at $-10°$ C. may remain active for years Many reports have appeared on the effect of various chemical substances on vaccinia virus, but the results vary with the nature and the reaction of the suspending medium, the amount of protective protein present and the temperature at which tests are made The virus loses activity at pH 3 0 within an hour It withstands the action of 1.0 per cent phenol for weeks at 4° C. but at 37° C may be inactivated in 24 hours It is not destroyed by certain disinfectants in concentrations which are rapidly lethal to most bacteria, e g, 1:10,000 brilliant green, but is inactivated by oxidizing disinfectants, e g, potassium permanganate 1·10,000 Alcohol, methyl alcohol and acetone in 50 per cent concentration inactivate vaccine lymph within 1 hour Vaccinia is more resistant to the action of ethyl ether than many other viruses Many substances have been tested for chemotherapeutic effect in vaccinial infected animals, but spectacular results have not been achieved (Chap 6).

Animals which have recovered from infection with vaccinia virus are resistant to further infection, and their sera will confer passive protection on normal animals against vaccinia virus The antibodies in the serum of immune animals may be increased by further injections of virus, and for the preparation of hyperimmune sera in rabbits purified suspensions of active elementary bodies may be injected intravenously. Injection of virus inactivated by heat or formalin into normal animals produces a relatively low degree of immunity to infection, and the sera of such animals has little protective or neutralizing effect on the virus (Parker and Rivers, 1936b) Inoculation of relatively large doses of purified virus inactivated by ultraviolet light produces a rather greater degree of immunity but is inferior to active virus as an immunizing agent (Collier et al, 1955) The sera of immune animals can be shown by various in vitro technics to react with virus preparations, indeed, precipitation and complement-fixation reactions were demonstrated many years ago (Smadel, 1952) By studies with purified suspensions of elementary bodies a picture of the antigenic structure of vaccinia has been built up as detailed as that available for many bacteria The following antigens have been recognized, LS or soluble antigen, NP or nucleoprotein antigen and a hemagglutinin In addition to the antibodies which react in vitro with these antigens, antivaccinial sera contains other antibodies which are considered below.

The LS antigen of vaccinia may be obtained free from virus in extracts of vaccinia-infected tissue and forms part of the surface of elementary bodies from which it slowly dissociates when purified elementary body suspensions are kept at refrigerator temperature The LS antigen flocculates and fixes complement when mixed with antivaccinial serum The agglutination of elementary bodies with specific antiserum is due, in part at least, to the presence of this antigen at the surface of the virus particles. Injections of LS antigen into rabbits stimulates antibody formation which reacts with the antigen in vitro, but the animals are not thereby immunized against infection with vaccinia virus nor do their sera contain neutralizing antibodies The L part of the antigen is inactivated by heating at

sence of antibody response, had a normal amount of gamma globulin in his serum The agammaglobulinemic infant suffering from progressive vaccinia recorded by Lewis and Johnson (1957) died, although large doses of immune gamma globulin had been given

Epidemiology

Accidental infection with vaccinia may occur in persons working in laboratories where the virus is being studied, and infection may be contracted through contact with a recently vaccinated person This is particularly liable to happen to individuals suffering from chronic dermatitis

Control Measures

Persons who propose to work with vaccinia should be vaccinated or revaccinated before beginning such work Children suffering from any form of chronic dermatitis should be protected from contact with recently vaccinated persons, where such exposure has occurred or when vaccination of such children has to be carried out because of the risk of exposure to smallpox, serious complications may be avoided by the administration of 0 6 ml of hyperimmune vaccinal gamma globulin per Kg body weight (Kempe et al, 1956)

REFERENCES

(For complete references, see also 2nd edition, chapter 19)

American Public Health Association, 1955, Smallpox *in* The Control of Communicable Diseases, ed 8, pp 164-167, New York, Am Pub Health A

Bancroft, I R, 1904, Clinical observations on variola, J Med Res *11*, 322-344

Barbero, G J, Gray, A, McNair-Scott, T F, and Kempe, H C, 1955, Vaccinia gangrenosa treated with hyperimmune vaccinal gamma globulin, Pediatrics *16*, 609-618

Bauer, D J, 1955, The antiviral and synergic actions of Isatin Thiosemicarbazone and certain Phenoxypyrimidines in vaccinia infection in mice, Brit J Exper Path *36*, 105-114

Becker, F T, 1940, Milkers' nodules, J A M A *115*, 2140-2143

Benenson, A S, Kempe, C H, and Wheeler, R E, 1952, Problems in maintaining immunity to smallpox, Am J Pub Health *42*, 535-541

Berger, K., 1955, Infektionsversuche mit dem Virus des Melkerknotens, Zentralbl Bakt (Abt 1) *163*, 365-372

———, 1956, Kuhpockenvirus und Vaccine virus, Ztschr Hyg *143*, 151 158

Bigler, J A, and Slotkowski, E L, 1951, Smallpox vaccination with prolonged vaccinia, Pediatrics *7*, 24-33

Bland, J O W, and Robinow, C F, 1939, The inclusion bodies of vaccinia and their relationship to the elementary bodies studied in cultures of the rabbit's cornea, J Path & Bact *48*, 381-403

Blaxall, F R, 1930, Smallpox *in* A System of Bacteriology in Relation to Medicine, vol 7, pp 84-132, London, His Majesty's Stat Off

Bohls, S W, and Irons, J V, 1942, Chorio-allantoic membrane infection as a diagnostic test for smallpox, Am J Pub Health *32*, 300-306

Bras, G, 1952, The morbid anatomy of smallpox, Docum med geog et trop *4*, 1-48

Brinckerhoff, W R, and Tyzzer, E E., 1906, Studies upon experimental variola and vaccinia in Quadrumana, J Med Res *14*, 213-359

Briody, B A, 1951, A unitarian view of vaccinia hemagglutinin, Yale J Biol & Med *24*, 1-4

Broom, J C, 1947, Revaccination in adults, Lancet *1*, 364-366

Buddingh, G J, 1938, Infection of the chorio-allantois of the chick embryo as a diagnostic test for variola, Am J Hyg *28*, 130-137

———, 1943, The pathogenic and antigenic properties of dermal vaccine virus propagated in the chorio-allantois of chick embryos, Am J Hyg *38*, 310-322

Buddingh, G J, and Randall, C C, 1951, Further studies on the preparation of smallpox vaccine by the chick-embryo method, Am J Hyg *53*, 152-163

Burnet, F M, and Stone, J D, 1946, The haemagglutinins of vaccinia and ectromelia viruses, Australian J Exper Biol & M Sc *24*, 1-8

Cabasso, V J, and Moore, I F, 1957, A proposed potency test for smallpox vaccine I Evaluation of chick embryo LD_{50} method Proc Soc Exper Biol & Med *95*, 605-609

Cassel, W A, 1957, Multiplication of vaccinia virus in the Ehrlich ascites carcinoma, Virology *3*, 514-526

Ceely, R, 1840 Observations on the variolae vaccinae, Tr Provincial Med & Surg A vol 8, reprinted *in* History and Pathology of Vaccination, vol 2, pp 365-443, E M Crookshank, 1889, London, Lewis

Chu, C M, 1948, Studies on vaccinia haemagglutinin I Some physiochemical properties J Hyg *46*, 42-48

Cockburn, W C, Cross, R M, Downie, A W, Dumbell, K R, Kaplan, C, McClean, D, and Payne, A M-M, 1957, Laboratory and vaccination studies with dried smallpox vaccines, Bull World Health Organ *16*, 63-77

Collier, L H, 1955, The development of a stable smallpox vaccine, J Hyg *53*, 76-101

Collier, L H, McClean, D, and Vallet, L, 1955, The antigenicity of ultraviolet irradiated vaccinia virus, J Hyg *53*, 513-534

Collier, W A., Smit, A M, and van Heerde, A F, 1950, Der Nachweis von Antihämagglutininen bei Variolapatienten als diagnostisches Hilfsmittel, Ztschr Hyg *131*, 555-567

pox and ectromelia show serologic similarity when cross-tested by the complement-fixation or inhibition of complement-fixation technics with immune sera prepared in rabbits or fowls (Downie and Macdonald, 1950) By neutralization tests with such sera on the chorio-allantois, the viruses of alastrim and variola could not be distinguished, and the immunologic differences between them and vaccinia, cowpox and ectromelia were no greater than the differences observed between some strains of influenza A virus (Downie and McCarthy, 1950).

Many substances have been tested for possible chemotherapeutic effect on vaccinia virus. Screening tests have usually been made for inhibitory effect on the growth of vaccinia virus in tissue culture or on infections of the chick embryo, mice or rabbits. Results have varied according to the technic used, so that a substance which may inhibit the virus in tissue culture may not influence the progress of infection in the chick embryo or the mouse. Sulfonamides and the antibiotics have generally little inhibitory effect. In the hope of interfering with the synthesis of virus nucleic acid or virus protein, analogues of purines, pyrimidines and amino acids have been examined Structural analogues of purine and pyrimidine bases may inhibit the growth of virus in tissue culture. Certain 5 phenoxy thiouracils, although only slightly inhibitory to vaccinia virus in the chick embryo, were found to have significant protective effect in mice (Thompson et al, 1951). Thiosemicarbazones also are reported to have some inhibitory activity in mice (Hamre et al, 1951; Bauer, 1955) and a series of benzimidazole derivatives suppressed vaccinial infection in the chorio-allantois in vitro (Tamm and Overman, 1957) The selective activity of different benzimidazole derivatives on vaccinia and influenza viruses appeared to be related to the different kind of nucleic acid in these 2 viruses None of these substances or any others tested so far seems to hold out any immediate prospect of practical application as therapeutic agents in diseases due to pox viruses. Recent reviews of this field are to be found in the paper of Horsfall and Tamm (1957) and in Chapter 6.

The growth of vaccinia virus has been followed in tissue culture, in the chick embryo, in the mouse and in the rabbit. Generally it has been found that increase of virus is first detectable after 8 to 16 hours from the time of inoculation, and thereafter there appears to be a logarithmic increase for the next 24 to 36 hours A variable decrease in infectivity has usually been noted during the first 8 to 10 hours after inoculation—the latent period On analogy with what has been observed with phage and reported also in the case of influenza virus, it has been suggested that vaccinia virus passes through a noninfective phase early in its intracellular development before mature particles are formed. Although some observers have reported an almost complete disappearance of infective virus during the first few hours, in the experiments of Overman and Tamm (1957) and of Maitland and Magrath (1957) the drop in infectivity amounted to only 50 per cent. In work of this kind the apparent loss of virus may be due to imperfection of methods for extracting infective virus from the tissues under examination. Although many of the reported studies on the eclipse phase support the view that there is an intracellular noninfective stage in the development of vaccinia virus, further work is desirable, perhaps with improved cell culture technics, before this view of the mode of growth of vaccinia virus could be generally accepted

Diagnosis

The diagnosis of the generalized forms of vaccinia will usually be made on the history of recent vaccination or contact with a recently vaccinated person and the character of the cutaneous lesions Where laboratory confirmation is required as, for example, for differential diagnosis of eczema herpeticum and eczema vaccinatum, the laboratory procedures outlined in the section on diagnosis of variola are applicable.

Treatment

In cases of generalized vaccinia where the immunologic response to infection may be deficient, hyperimmune vaccinal gamma globulin may be of value (Kempe et al, 1956). Of the cases of progressive generalized vaccinia recorded, the only one which recovered received 5 injections of immune gamma globulin over a period of 6 weeks (Barbero et al, 1955). But this patient, in spite of the ab-

Helbert, D, 1957, Smallpox and alastrim Use of the chick embryo to distinguish between the viruses of variola major and variola minor Lancet *1*, 1012-1014

Herrlich, A, 1954, Probleme der Pocken und Pockenschutzimpfung, Munch med Wchnschr *96*, 529-533

Herrlich, A, and Mayr, A, 1954, Vergleichende experimentelle Arbeiten uber die Vaccine-Kuhpockenviren, Arch Hyg *138*, 479-504

———, 1955, Die Differenzierung der Tierpockenviren im bebruteten Huhnerei, Arch Hyg *139*, 444-466

Herzberg, K., 1955, Vergleichende Vaccinevirusuntersuchungen, Zentralbl Bakt (Abt 1), *162*, 405-430

Hester, H R., Boley, L E, and Graham, R, 1941, Studies on cowpox I An outbreak of natural cowpox and its relation to vaccinia Cornell Veterinarian *31*, 360-378

Himmelweit, F, 1938, Observations on living vaccinia and ectromelia viruses by high power microscopy, Brit J Exper Path *19*, 108-123

Hooker, S B., 1929, A skin test for susceptibility to smallpox, human endermal reactions to killed vaccine virus, J Infect Dis *45*, 255-262

Horsfall, F L, and Tamm, I, 1957, Chemotherapy of viral and rickettsial diseases, Ann Rev Microbiol *11*, 339-370

Illingworth, R S, and Oliver, W A, 1944, Smallpox in the Middle East lessons from 100 cases, Lancet *2*, 681-685

Jackson, E B, Ley, A C, Binn L N and Smadel, J E., 1956, Vaccination against smallpox I Lyophilized vaccine virus from infected chorioallantoic membranes J Immunol *77*, 332-339

Jenner, E., 1798, An inquiry into the causes and effects of the variolae vaccinae, a disease discovered in some of the western counties of England, particularly Gloucestershire, and known by the name of the cow pox Reprinted by Cassell & Company, Limited, 1896 Available in Pamphlet Vol 4232 Army Medical Library, Washington, D C

Jong, M de, 1956, The alastrim epidemic in The Hague 1953 54, Docum med geog et trop *8*, 207-235

Kaidan, S E, McCarthy, K., and Haworth J C, 1953, Fatal generalized vaccinia with failure of antibody *production and absence of serum gamma* globulin, Arch Dis Children *28*, 110-116

Kempe, C H, 1956 Variola and vaccinia in Diagnostic Procedures for Virus and Rickettsial Diseases, ed *2, pp 341 361*, New York, Am Pub Health A

Kempe, C H, Berge, T O, and England, B, 1956, Hyperimmune vaccinal gamma globulin Source, evaluation and use in prophylaxis and therapy Pediatrics *18*, 177-188

Laidlaw, S I A, and Horne, W A, 1950 Smallpox outbreak in Glasgow, 1950, M Officer *83*, 187-192

Lazarus, A S, Eddie, B, and Meyer, K F, 1937, Propagation of variola virus in the developing egg, Proc Soc Exper Biol & Med *36*, 7-8

Lea, D E, and Salaman, M H., 1942, The inactivation of vaccinia virus by *radiations*, Brit J Exper Path *23*, 27-37

Lewis, H M., and Johnson, F C., 1957, Fatal agammaglobulinemic progressive vaccinia Report of a case with a classification *of human cutaneous* vaccinia A M A Arch Dermat *75*, 837-844

Lynch, F W., 1932, Dermatologic conditions of the foetus with particular reference to variola and vaccinia, Arch Dermat Syph *26*, 997-1019

MacCallum, F O, 1954, Early diagnosis of smallpox in the Dynamics of Virus and Rickettsial Infections, pp 324-333, New York, Blakiston

MacCallum, F O, and McDonald, J R., 1957, Survival of variola virus in raw cotton, Bull World Health Organ *16*, 247-254

MacCallum, F O, McPherson, C A, and Johnstone, D F, 1950, Laboratory investigation of smallpox patients with particular reference to infectivity *in the early stages*, Lancet *2*, 514-517

McCarthy, K, and Downie, A W, 1953, The serum antibody response in alastrim, Lancet *1*, 257-260

Magrath, G B, and Brinckerhoff, W R, 1904, On the occurrence of *Cytoryctes variolae*, Guarnieri, in the skin of the monkey inoculated with variola virus, J Med Res *11*, 173-179

Maitland H B, and Magrath, D I, 1957, The growth in vitro of vaccinia virus in chick embryo chorio-allantoic membrane, minced embryo and cell suspensions, J Hyg *55*, 347-360

Marsden, J P., 1948, Variola minor A personal analysis of 13,686 cases Bull Hyg *23*, 735-746,

Mayr, A, Herrlich, A, and Mahnel, H, 1955, Experimentelle untersuchungen uber das S antigen bei den Tierpockenviren, Arch Hyg *139*, 580-607

Morgan, C., Ellison, S A, Rose, H M, and Moore, D H, 1954, Structure and development of viruses observed in the electron microscope II Vaccinia and fowl pox viruses J Exper Med *100*, 301-310

Murray L H, 1951, A World review of smallpox incidence, Epidemiol & Vital Statist Rep *4*, 398-420

Nagler, F P O, 1942, Application of Hirst's phenomenon to the titration of vaccinia virus and vaccinia immune serum, M J Australia *1*, 281-283

Nagler, F P O, and Rake, G, 1948, The use of the electron microscope in diagnosis of variola, vaccinia, and varicella, J Bact *55*, 45-51

National Institutes of Health, 1946, Minimum requirements smallpox vaccine (2nd revision), Bethesda, Maryland, Nat Insts Health

Nelson, J B, 1939, Behaviour of pox viruses in respiratory tract response of mice to nasal instillation of variola virus, J Exper Med *70*, 107-116

———, 1943 The stability of variola virus propagated in embryonated eggs, J Exper Med *78*, 231-239

Nomland, R, and McKee, A P, 1932, Milkers' nodules, report of ten cases, Arch Dermat Syph *65*, 663-672

North, E A, 1944, A study of the immunological reactions of the variola and vaccinia viruses grown in the developing egg, Australian J Exper Biol & M Sc *22*, 105-109

Cook, E B M, Bell, B, Forsyth, P, Irons, J V, and Cox, G W, 1953, Use of chorio-allantoic membrane culture smallpox vaccine in Texas, Texas Rep Biol & Med *11*, 522-529
Councilman, W T, Magrath, G B, and Brinckerhoff, W R, 1904, The pathological anatomy and histology of variola, J Med Res *11*, 12-135
Craigie, J, 1932, The nature of the vaccinia flocculation reaction, and observations on the elementary bodies of vaccinia, Brit J Exper Path *13*, 259-268
Craigie, J, and Wishart, F O, 1936a, The complement-fixation reaction in variola, Canad J Pub Health *27*, 371-379
———, 1936b, Studies on the soluble precipitable substances of vaccinia II The soluble precipitable substances of dermal filtrate J Exper Med *64*, 819-830
Cramb, R, 1951, Smallpox outbreak in Brighton, 1950-51, Pub Health *64*, 123-128
Creighton, C, 1894, A History of Epidemics in Britain, vol 2, pp 434-631, London, Cambridge Univ Press
Dawson, I M, and McFarlane, A S, 1948, Structure of an animal virus, Nature, London *161*, 464-466
Dekking, F, 1950, Koepokken en vaccinia, Tidschrift voor Diergeneeskunde *75*, 248-250
———, 1955, Personal communication
Dinger, J E, 1956, Difference in persistence of smallpox and alastrim virus on the chorio-allantois, Docum med geog et trop *8*, 202-206
Dixon, C W, 1948, Smallpox in Tripolitania, 1946 An epidemiological and clinical study of 500 cases including trials of penicillin treatment J Hyg *46*, 351-377
Downie, A W, 1939a, A study of the lesions produced experimentally by cowpox virus, J Path & Bact *48*, 361-379
———, 1939b, The immunological relationship of the virus of spontaneous cowpox to vaccinia virus, Brit J Exper Path *20*, 158-176
Downie, A W, and Dumbell, K R, 1947, The isolation and cultivation of variola virus on the chorio-allantois of chick embryos, J Path & Bact *59*, 189-198
———, 1956, Pox viruses, Ann Rev Microbiol *10*, 237-252
Downie, A W, and Haddock, D W, 1952, A variant of cowpox virus, Lancet *1*, 1049-1050
Downie, A W, and McCarthy, K, 1950, The viruses of variola, vaccinia, cowpox and ectromelia Neutralization tests on the chorio-allantois with unabsorbed and absorbed immune sera Brit J Exper Path *31*, 789-796
D[illegible]
Lancet 2, 104-106
Downie, A W, and Macdonald, A, 1950, A study of the pox viruses by complement-fixation and inhibition of complement fixation methods, J Path & Bact *62*, 389-401
———, 1953, Smallpox and related virus infections of man, Brit M Bull *9*, 191-195.
Dumbell, K R, Downie, A W, and Valentine, [redacted] C, 1957, The ratio of the number of virus particles to infective titer of cowpox and vaccinia virus suspension, Virology *4*, 467-482
Eagles, G H, and Ledingham, J C G, 1932, Vaccinia and the Paschen body infection experiments with centrifugalized virus filtrates, Lancet *1*, 823-826
Easton, J H L, 1945, An outbreak of smallpox in the Middle East, Pub Health *58*, 110-114
Elford, W J, and Andrewes, C H, 1932, Filtration of vaccinia virus through gradocol membranes, Brit J Exper Path *13*, 36-42
Fabre, J, 1948, Smallpox prevalence throughout the World during and after the Second World War, Epidemiol & Vital Statist Rep *1*, 268-289
Fenner, F, 1958, The biological characters of several strains of vaccinia, cowpox and rabbitpox viruses, Virology *5*, 502-529
Gaylord, W H, and Melnick, J L, 1953, Intracellular forms of pox viruses as shown by the electron microscope (vaccinia, ectromelia and molluscum contagiosum), J Exper Med *98*, 157-172
Gear, H S, and Deutschman, Z, 1956, Disease Control and International Travel A review of the International Sanitary Regulations Smallpox, pp 27-31 Geneva, World Health Organ
Gillen, A L, Burr, M M, and Nagler, F P, 1950, Recovery of two distinct hemagglutinins of vaccinia virus, J Immunol *65*, 701-706
Gispen, R, 1952, Silver impregnation of smallpox elementary bodies after treatment with xylol, Antonie van Leeuwenhoek *18*, 107-108
———, 1955, Analysis of pox-virus antigens by means of double diffusion A method for direct serological differentiation of cowpox J Immunol *74*, 134-141
Goodpasture, E W, Woodruff, A M, and Buddingh, G J, 1932, Vaccinial infection of the chorio-allantoic membrane of the chick embryo, Am J Path *8*, 271-282
Gordon, M H, 1925, Studies of the viruses of vaccinia and variola Med Res Council, Special Report Series No 98, London, His Majesty's Stat Off
———, 1937, Virus bodies John Buist and the elementary bodies of vaccinia, Edinburgh M J *44*, 65-71
Green, R H, Anderson, T F, and Smadel, J E, 1942, Morphological structure of the virus of vaccinia, J Exper Med *75*, 651-656
Greenberg, M, 1948, Complications of vaccination against smallpox, Am J Dis Child *76*, 492-502
Hall, G F M, Cunliffe, A C, and Dudgeon, J A, 1953, Prolonged generalized vaccinia, J Path & Bact *66*, 25-38
[illegible]
II The activity of some thiosemicarbazones J Immunol *67*, 305-312
Haygarth, J, 1793, *A Sketch of a Plan to Exterminate the Casual Smallpox from Great Britain*, vol 1, pp 138-143, London, Johnson

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33

Psittacosis—Lymphogranuloma Venereum Group

INTRODUCTION

These large intracellular parasites (1) responsible for diverse, natural, clinical and latent infections in birds, man and other mammals, (2) readily stained by basophilic dyes, (3) antigenically related, according to complement fixation, cross immunity and toxin neutralization tests, (4) producing pneumonitis in the laboratory mouse when introduced by the intranasal route, (5) growing well in the yolk sac of the embryonated egg and (6) susceptible to the action of certain chemotherapeutic agents form a distinct biologic group. Since the basic group characteristics were first recognized in the agent responsible for psittacosis and several years later in that of lymphogranuloma venereum, the group is called the psittacosis-lymphogranuloma venereum group. It is composed of a large, increasing number of similar infectious agents whose nature and relationship to other micro-organisms is still imperfectly understood. In susceptibility to sulfonamides, penicillin, tetracycline and other chemotherapeutic agents they resemble the bacteria, in their strictly intracellular multiplication with cytoplasmic inclusion bodies and in their relationship to the metabolism of animal cells they behave more like true viruses than like rickettsiae. Some of their morphologic and antigenic properties relate them to the newly created group of Neorickettsiae (Philip et al., 1953, Philip, 1955, Giroud and Jadin, 1954, Giroud et al., 1955, Giroud, 1956). They lie along an indistinct line that separates virus from not virus. It is too soon to follow the taxonomy proposed by Moshkovsky, 1945, who suggested the family name *Chlamydozoaceae* and the genus name *Miyagawanella*. The group is now categorized on the basis of host-parasite relationships; the ultimate classification probably will be based on antigenic characteristics. Since Bedson (1936) made the first comprehensive study of a member of this group, including identification of 2 antigens of the psittacosis agent, it may be appropriate to consider *Bedsonia* as the name of the genus, at least for all of the parasites now called psittacosis viruses of avian, human and mammalian origin (Meyer, 1953). If the serologically related viruses were to be placed in a single genus, then the 8 species given in Bergey's *Manual* require redistribution.

For nearly 10 years after the first isolation of psittacosis virus from parrots and patients in 1930 it was believed that *Psittaciformes* imported from their natural tropical habitat or raised by hobbyists or commercially in the United States and Europe were the only sources of the virus. Then fulmars, or petrels, were found to be sources of hu-

Otten, L, Collier, W A, and Meyer, F H, 1950, Dry lymph vaccine (Pub 27), Organ Scientif Res in Indonesia, Djakarta, pp 1-32

Overman, J R, and Tamm, I, 1956, Equivalence between vaccinia particles counted by electron microscopy and infectious units of the virus, Proc Soc Exper Biol & Med *92*, 806-810

———, 1957, Multiplication of vaccinia virus in the chorio-allantoic membrane in vitro, Virology *3*, 173-184

Parker, R F, and Rivers, T M, 1936a, Immunological and chemical investigations of vaccine virus IV Statistical studies of elementary bodies in relation to infection and agglutination J Exper Med *64*, 439-452

———, 1936b, Immunological and chemical investigations of vaccina virus III Response of rabbits to inactive elementary bodies of vaccinia and to virus-free extracts of vaccine virus J Exper Med *63*, 69-94

Paschen, E, 1924, Die Pocken *in* G Jochmann's Lehrbuch der Infektionskrankeiten, ed 2, pp 843-899, Revised by C Hegler, Berlin, Springer

Peters, D, and Nasemann, T, 1952, Untersuchungen am Virus Variola-Vaccine 1 Uber den wert der Tupfpreparat fur die elektronenoptische Abbildung und Ausmassung von Elementerkörpen Ztschr Tropenmed u Parasitol *4*, 11-25

Peters, D, and Stoeckenius, W, 1954, Untersuchungen am Virus der Variola-Vaccine III Mitt Enzymatischer Abbau des Innenkörpers Ztschr f Naturforsch *9b*, 524-529

Report, 1957, Smallpox in Europe during the ten last years, Epidemiol & Vital Statist Rep *10*, 147

Ricketts, T F, and Byles, J B, 1908, The Diagnosis of Smallpox, 154 pp, London, Cassell

van Rooyen, C E, and Illingworth, R S, 1944, A laboratory test for diagnosis of smallpox, Brit M J *2*, 526-529

van Rooyen, C E, and Rhodes, A J, 1948, The variola-vaccinia virus animal experiments *in* Virus Diseases of Man, pp 308-333, New York, Nelson

van Rooyen, C E, and Scott, G D, 1948, Smallpox diagnosis with special reference to electron microscopy, Canad J Pub Health *39*, 467-477

Ryden, F W, and Randall, C C, 1957, The growth cycle of vaccinia in HeLa cells correlated with concurrent cellular changes, Am J Path *33*, 357-383

Salaman, M H, 1937, The combining properties of vaccinia virus with the antibodies demonstrable in anti-vaccinial serum, Brit J Exper Path *18*, 245-258

Smadel, J E, 1952, Smallpox and vaccinia *in* Viral and Rickettsial Infections of Man, ed 2, pp 414-439, Philadelphia, Lippincott.

Smadel, J. E, Rivers, T. M, and Hoagland, C L., 1942, Nucleoprotein antigen of vaccine virus I. A new antigen obtained from elementary bodies of vaccinia Arch Path *34*, 275-285

Smadel, J. E, Rivers, T M, and Pickels, E G., 1939, Estimation of the purity of preparations of elementary bodies of vaccinia, J Exper Med *70*, 379-385

Smadel, J E, and Shedlovsky, T, 1942, Antigens of vaccinia, Ann New York Acad Sc *43*, 35-46

Smadel, J E, and Wall, M J, 1937, Elementary bodies of vaccinia from infected chorio-allantoic membranes of developing chick embryos, J Exper Med *66*, 325-336

Sommerville, J, Napier, W, and Dick, A, 1951, Kaposi's varicelliform eruption record of an outbreak, Brit J Dermat *63*, 203-214

Stallybrass, C O, 1931, The Principles of Epidemiology and the Process of Infection, pp 329, 338, London, Routledge

Stoeckenius, W, and Peters, D, 1955, Untersuchungen am Virus der Variola-Vaccine IV Mitt Die morphologie des "Innenkorpers" Ztschr f Naturforsch *10b*, 77-80

Stuart, G, 1947-1948, Memorandum on post-vaccinal encephalitis, Bull World Health Organ *1*, 36-53

Sweitzer, S E, and Ikeda, K, 1927, Variola, a clinical study of the Minneapolis epidemic of 1924-1925, Arch Dermat. Syph *15*, 19-29

Tamm, I, and Overman, J. R, 1957, Relationship between structure of benzimidazole derivatives and inhibitory activity on vaccinia virus multiplication, Virology *3*, 185-196

[illegible] Falco,

491

van Tongeren, H A E, 1952, Spontaneous mutation of cowpox-virus by means of egg passage Arch ges Virusforschung *5*, 35-52

Torres, C M, 1935-1936, Further studies on the pathology of alastrim and their significance in the variola-alastrim problem, Proc Roy Soc Med *29*, 1525-1539

Torres, C M, and Teixeira, J de C, 1935, Culture de virus de l'alastrim sur les membranes de l'embryon de poulet, Compt Rend Soc Biol *118*, 1023-1024

Verlinde, J D, 1951, Koepokken bij de mens, Tijdschrift voor Diergeneeskunde *76*, 334-342

Waldo, H H, 1955, Smallpox among "vaccinated" troops, U S Armed Forces M. J. *6*, 799-806

Wesslen, T, 1955, The production of smallpox vaccine in tissue culture of bovine embryonic skin, Arch ges Virusforschung *6*, 430-438

Woodville, W., 1796, The History of the Inoculation of the Smallpox in Great Britain, vol 1, sect 4, pp 153-228, London, James Phillips

Wyckoff, R W. G, 1951, The virus of vaccinia in chick embryo membrane, Proc Nat Acad Sc *37*, 565-569

PSITTACOSIS

(SYNONYMS: Parrot fever, ornithosis, *psittacose*, *Papageienkrankheit*)

INTRODUCTION

In his thesis, *De la psittacose ou infection spéciale déterminée par des perruches*, Antonin Morange introduced the name psittacosis for a clinically defined disease of man caused by parrots, and because of its appropriateness to what was then known of the infection, this term found wide usage When it was recognized that a great variety of birds are affected by this virus, the more general term "ornithosis" was introduced The word "psittacosis' refers to a generalized viral infection of man derived from birds, among which parrots, parakeets, lovebirds, ducks pigeons and turkeys are important sources, and to the infections caused naturally by the same agents in psittacine birds Ornithosis regarded as an equivalent term in the sixth revision of the *International List of Diseases and Causes of Death* and as a synonym in the eighth edition of *Control of Communicable Diseases in Man* (1955), is reserved for infections of similar etiology in extrapsittacine birds

The human illness may be severe carrying a high mortality rate, or it may be mild or even subclinical It affects all ages and both sexes Its cause is an elementary body virus that develops in reticuloendothelial cells

HISTORY

From a relatively obscure disease first recognized in Switzerland, then in France and Germany, psittacosis became the subject of world-wide interest in 1929 and 1930 when outbreaks occurred in 12 countries and involved about 800 people Careful inquiries by Roubakine, 1930, and by Barros, 1940, indicate that parrots shipped out from South America were the main source of the infection (Meyer, 1942, Van Rooyen 1955) The eventual isolation of the agent from parakeets, pigeons, doves, chickens, ducks, seagulls, turkeys and other birds (Meyer and Eddie, 1952) has well established that there were sources of this infection independent of imported exotic birds Levinthal, Coles and Lillie in 1930 simultaneously discovered the minute spherical bodies within reticuloendothelial cells, and Bedson and Bland (1932) conclusively proved the etiologic relationship of the elementary bodies to infection Propagation of the virus in embryonated eggs and tissue cultures has furnished suitable antigens for serologic studies, and methods of isolating the viral agent from the sputum and the blood of psittacosis patients have been developed Procedures for measuring anti-infectious and toxin-neutralizing properties have revealed the antigenic relationship of strains isolated from birds and mammals. Wide-spectrum antimicrobial drugs tested experimentally and clinically have reduced the case fatality rate in man and have furnished promising evidence that chemotherapy may be used to render psittacine birds noninfectious (Cox, 1955)

CLINICAL PICTURE

The clinical form characterized by pneumonic consolidation and a mortality rate of 20 per cent was the first to be described in the classic accounts of the French clinicians, but now it is known that the severity varies within wide limits Serologic studies indicate that subclinical infections occur, and because of their similarity to common respiratory disease it is fairly certain that mild, atypical and ambulatory infections often go unrecognized Moderately severe, severe and fatal infections are in all probability less frequent but they are more commonly diagnosed because their cause is sought The duration of the illness may be short, terminating abruptly, or it may pursue a low-grade course over 3 months.

After an incubation time of 1 or 2 weeks, occasionally 3 or 4 weeks, the onset may be sudden with chilly sensations, fever, anorexia sore throat, malaise, photophobia and severe headache, or it may be gradual and insidious. The temperature at the onset is usually from 100° to 102° F and gradually rises During the second week in severe cases it remains high with slight morning remissions; in mild cases it may fall to normal on the 7th or 8th day. The pyrexia subsides by lysis

On physical examination of the thorax, moist rales in the absence of consolidation is the most characteristic finding It is not unusual, however, that there are no abnormal findings or that there is consolidation. The extent of the pneumonitis is usually not evi-

man infections on the Faeroe Islands In that year lymphogranuloma venereum found its place in the psittacosis group when the large elementary bodies in mononuclear cells of tissues were obtained from afflicted human beings, grew in the embryonated chicken egg and revealed a developmental cycle very similar to that of psittacosis The study of certain forms of pneumonia in human beings and of the lungs or other tissues of healthy or diseased mammals soon led to the discovery of viral agents not readily distinguishable from those causing psittacosis Not only are viruses of this group being recognized as the cause of disease in a growing number of mammals, but also similar infections are being found in many extrapsittacine species. The epidemiologic problems are multiplying accordingly. Little is yet known about the mammalian viruses or about their incidence in domestic animals or man They have caused significant economic losses of domestic herbivores Widespread latent infections in wild and domestic pigeons and barnyard fowl such as chickens, ducks and particularly turkeys, correctly designated ornithosis, can serve as reservoirs for serious and fatal "zoo-anthroponosis." Epizootics induced by vigorous, virulent strains impose heavy direct and indirect losses on the poultry industry

The psittacosis-lymphogranuloma venereum agents isolated during the past 20 years, grouped according to their host, are

1. **Avian Viruses.** Psittacosis virus (Coles, 1930, Levinthal, 1930, Lillie, 1930), ornithosis viruses including fulmar virus (Haagen and Mauer, 1938), pigeon and dove virus (Coles, 1940, Meyer and Eddie, 1941),

wycz et al, 1955), pheasant virus (Meyer and Eddie, 1956b)

sum viruses A and B (... 1949), bovine enteritis (York and Baker, 1956), bovine encephalomyelitis virus (Menges et al, 1953), enzootic abortion of ewes virus (Stamp et al, 1950; Barwell and Bishop, 1951), pneumonitis of sheep (McKercher, 1952), pneumonitis of goats (Omori et al, 1953), bovine pneumonia virus (Matumoto et al, 1955).

3. **Human Pneumonitis Viruses:** The high person-to-person communicability is the basis of the idea that the San Francisco (S F.) virus (Eaton et al., 1941), Louisiana (Borg) virus (Olson and Treuting, 1944), Chicago virus (Zichis and Shaughnessy, 1945) and pneumonitis virus (Yeatman and McEwin, 1945; de Gara and Furth, 1948) are specifically human strains

The host-parasite relationship of the viruses of this group is founded in the unique biologic characteristic of residual quiescent infection or latency first demonstrated in birds, then mammals (e g, mice, cattle, sheep and goats) and finally in man As a rule, infection takes place in young birds and mammals. The mortality rates vary; epizootics sometimes occur in poultry flocks and aviaries, but in the natural habitat of wild birds they are exceptional A stable association of host and parasite appropriate for survival of both is recovery of the host followed by long-lasting subclinical infection, providing the parasite ample opportunity to infect other hosts but having virtually no deleterious effect on the host. When an epizootic leads to the death of a large proportion of hosts a breakdown in this established equilibrium has taken place The virus may infect some new susceptible species brought into its environment, or a virus of higher virulence may emerge as a result of repeated transfer through fully susceptible hosts. In a proportion of the infected, after benign illness inapparent infection persists; the virus present in reticuloendothelial cells may be confined indefinitely to the viscera, or it may spill over and leave the infected hosts in excreta ... able percentage of the in- ... vi- ... ues ... ost- ... va-

tion, crowding, low temperature, illness, possibly hormonal factors (Austin, 1957), the virus may multiply beyond the capacity of the fragile immunity and thus be released into the environment. The large reservoirs of virus in birds and mammals in nature are usually self-confined, but under conditions frequently not clearly understood the overflow of the infective agent endangers the health and the welfare of other hosts, including man.

exudate and the minor change in the large bronchioles and bronchi give the pneumonia in psittacosis a fairly characteristic, but not pathognomonic, pattern. In some cases the lesions are similar to those in interstitial pneumonia associated with other viruses. Binford and Hauser (1944) distinguished between the microscopic lung lesions of psittacosis and those of Q fever and other virus pneumonias.

In the slightly enlarged and congested liver, the characteristic microscopic lesions are due to focal necrosis. Kupffer cells contain elementary bodies. The spleen may or may not be enlarged and may contain relatively small follicles and engorged sinuses filled with phagocytic cells. Cloudy swelling, hypertrophy of the cardiac muscle, interstitial edema, infiltration with plasma cells, and lymphocytes and subendocardial hemorrhages in the region of the mitral, the aortic and the bicuspid valves have been reported. The parenchyma of the kidneys may be degenerated. Hemorrhages and capillary thrombi have been observed in the adrenals of patients infected with the highly toxic Louisiana virus and in the glomerular capillary tufts of the frontal lobes of the brain (Prouty and Jordan, 1956). Congestion and edema of the brain and the spinal cord are not infrequent. Chromatolysis in the anterior horn cells, glia proliferation, occasional changes in the ganglion and proliferative degenerative changes in the capillary endothelium are attributable to toxic factors primary or secondary to pneumonia. In the rare cases of meningitis intracytoplasmic inclusions have been identified in the gelatinous exudate over the pia arachnoid (Walton, 1954).

Two observations have been made early in experimental infection that may explain the pathogenicity of the elementary bodies. In murine pneumonitis of mice following aerosol exposure, throughout the development of the elementary bodies to vesicles no inflammatory reaction could be discerned in the alveoli. But when the vesicles burst, probably releasing toxin or host cell components, polymorphonuclear cells accumulated in the alveoli, lung macrophages were mobilized, and phagocytes poured out from the capillaries into the alveoli. The intense cellular infiltration interfered with further vesicle formation, and virus proliferation was never most active in the center of the inflammatory foci. When infection leads to complete consolidation of the lungs, death results from suffocation. The injury by murine pneumonitis virus is relatively direct and causes a nonspecific reaction in the host (Gogolak, 1953).

Experimental Infection, Host Range

Members of this group vary considerably in their affinity for tissue cells and hosts. The most virulent psittacosis and turkey ornithosis strains for man have the broadest range of host and tissue pathogenicity. They are followed in decreasing breadth of range by ornithosis strains isolated from pigeons and ducks, by meningopneumonitis, feline and mouse pneumonitis and other mammalian strains.

Mice, the most useful indicator animals, may be infected by the intranasal, intraperitoneal, intracerebral, intravenous or subcutaneous routes or by feeding, depending on the viral isolates used. They are of little value in isolating virus from cattle or goats. The duration of the illness depends on the amount, the virulence and the toxin-producing ability of the virus. Intravenous and intraperitoneal titrations in mice of isolates from birds have given useful information about their virulence. Repeated titrations reveal differences between log ID_{50} and LD_{50}, and these may be used to calculate a pathogenicity index. As the lethality titer approaches equivalence with the infectivity titer, the index approaches 0. A low index indicates high mouse pathogenicity, and a high index (i.e., 4) indicates an infectivity 10,000 times greater than the lethality. In general, isolates from severe human infections, parrots, parakeets and turkeys involved in human outbreaks have yielded a low index. Strains from pigeons and ducks and from sporadic infections in turkeys have yielded high indices.

Death ensues in 2 to 6 days for the highly virulent and in 8 to 15 days for the low virulent strains, some mice recover. In animals infected orally or subcutaneously the course is always protracted. Latent infections have persisted for 10 to 12 months. The virus has been isolated from mice treated with antimicrobial drugs as long as 277 days after inoculation (Hurst et al., 1953). At necropsy 2 to 4 days after intraperitoneal inoculation the spleen and the liver look normal, but the abdominal viscera are covered by a thin, sticky exudate consisting of endothelial cells

dent until roentgenologic examination, which shows infiltration. This may be patchy over *one or both lungs, but it is usually broncho*pneumonic. Physical signs begin to subside by the third week, but roentgenograms disclose a slower resolution. Pleural reaction is generally slight or absent. During the first few days a slight irritating cough may persist or increase, or, despite extensive lung involvement, cough may be insignificant or ab*sent throughout the entire illness. There is* usually no excess of sputum; in some cases it may be at first mucoid and later mucopurulent. The rate and the depth of respirations are not increased except in fatal cases, in which a rate as high as 60 per minute has been observed.

The relative slowness of the pulse is characteristic, but in nearly all fatal cases the pulse becomes rapid and weak. Cyanosis and low blood pressure may be marked; collapse *sometime during the illness is common. The* cardiovascular system is altered in many cases. Myocardial involvement, which may extend far into convalescence, is being observed oftener since electrocardiograms are more common (Lyon, 1956, Kemmerer et al, 1956) Epistaxis and rose spots are considered signs of general vascular damage. Thrombophlebitis *may occur and cause death* through pulmonary embolism (Jørgensen and Steffensen, 1956).

*Severe psittacosis shows the greatest vari*ability in clinical features. Nausea and vomiting are common, and either constipation or diarrhea may be present. *Albuminuria is not* infrequent. In some patients organs and sys*tems other than the lungs are involved.* Hepatosplenomegaly, even acute thyroiditis, have been reported (Seibert et al., 1956). Jaundice and oliguria have been noted (Yow et al., 1957). The spleen may be enlarged temporarily, but in most cases it is not palpable (Meyer, 1954). The leukocyte count is either normal or subnormal, with eosinopenia; leukopenia is pronounced in only about 25 per cent of the cases. Leukocytosis occurs late in the disease or in early convalescence. Psittacosis, if treated early and for at least *10 days, seems to run a smooth course,* and circulatory complications are exceptional. Inadequate treatment with respect both to dosage and to time *greatly favors relapses and* slow convalescence. Meningitis, encephalitic symptoms, insomnia, disorientation, apathy, mental depression and even delirium may occur in all except mild infections. In the terminal stages death generally results from pulmonary insufficiency and generalized toxemia.

Until quite recently clinical evidence of in*fection in children was considered unusual.* But in family outbreaks sometimes the children in intimate contact with infective material, such as feathers and droppings, while fondling parakeets or cleaning cages, have become ill or have had no symptoms but developed antibodies (Strobel, 1954; Lippelt and Brand, 1955; Babudieri and Cerri, 1956; *Prouty and Jordan, 1956). In Israel infants* from 1 month to 2½ years old contracted psittacosis. In 6 cases an ornithosis virus was isolated from infants with respiratory symptoms (Ephrati-Elizur and Bernkopf, 1956); 2 cases were fatal, 2 patients suffered moderate to severe respiratory disease, others had only a mild respiratory infection (Berman *et al., 1955).*

Pathologic Picture

... those of ... n in the ... user, *19*... idly palpable and sharply demarcated from normal lung tissue. They are gray, gray-red or plum-c... there ... posits. *In the trachea and the ...* cases they are empty, and the mucosa is not swollen. When the psittacotic process is com*plicated by secondary bacterial invasion, the* mucosa is swollen, and the bronchi are filled with purulent exudate. Microscopic examina*tion discloses that the portions that* had looked completely consolidated have undergone unevenly distributed lobular changes *Alveoli containing air or serum are dispersed* throughout the consolidated portion In fully developed lesions the alveolar spaces contain *abundant fibrin and many lymphocytes,* macrophages and desquamated alveolar epithelial cells Many cells in the alveolar exudate and *in the lymph sinuses of the hilar lymph nodes* show evidence of active phagocytosis and intracytoplasmic elementary bodies The scarcity of polymorphonuclear leukocytes in the

exudate and the minor change in the large bronchioles and bronchi give the pneumonia in psittacosis a fairly characteristic, but not pathognomonic, pattern In some cases the lesions are similar to those in interstitial pneumonia associated with other viruses Binford and Hauser (1944) distinguished between the microscopic lung lesions of psittacosis and those of Q fever and other virus pneumonias

In the slightly enlarged and congested liver, the characteristic microscopic lesions are due to focal necrosis Kupffer cells contain elementary bodies The spleen may or may not

the cardiac muscle, interstitial edema, infiltration with plasma cells, and lymphocytes and subendocardial hemorrhages in the region of the mitral, the aortic and the bicuspid valves have been reported The parenchyma of the kidneys may be degenerated Hemorrhages and capillary thrombi have been observed in the adrenals of patients infected with the highly toxic Louisiana virus and in the glomerular capillary tufts of the frontal lobes of the brain (Prouty and Jordan, 1956) Congestion and edema of the brain and the spinal cord are not infrequent Chromatolysis in the anterior horn cells, glia proliferation, occasional changes in the ganglion and proliferative degenerative changes in the capillary endothelium are attributable to toxic factors primary or secondary to pneumonia In the rare cases of meningitis intracytoplasmic inclusions have been identified in the gelatinous exudate over the pia arachnoid (Walton, 1954)

Two observations have been made early in experimental infection that may explain the pathogenicity of the elementary bodies In murine pneumonitis of mice following aerosol exposure, throughout the development of the elementary bodies to vesicles no inflammatory reaction could be discerned in the alveoli But when the vesicles burst, probably releasing toxin or host cell components, polymorphonuclear cells accumulated in the alveoli, lung macrophages were mobilized, and phagocytes poured out from the capillaries into the alveoli The intense cellular infiltration interfered with further vesicle formation, and virus proliferation was never most active in the center of the inflammatory foci When infection leads to complete consolidation of the lungs, death results from suffocation The injury by murine pneumonitis virus is relatively direct and causes a nonspecific reaction in the host (Gogolak, 1953)

Experimental Infection, Host Range

Members of this group vary considerably in their affinity for tissue cells and hosts The most virulent psittacosis and turkey ornithosis strains for man have the broadest range of host and tissue pathogenicity They are followed in decreasing breadth of range by ornithosis strains isolated from pigeons and ducks, by meningopneumonitis, feline and mouse pneumonitis and other mammalian strains

Mice, the most useful indicator animals, may be infected by the intranasal, intraperitoneal, intracerebral, intravenous or subcutaneous routes or by feeding, depending on the viral isolates used They are of little value in isolating virus from cattle or goats The duration of the illness depends on the amount, the virulence and the toxin-producing ability of the virus Intravenous and intraperitoneal titrations in mice of isolates from birds have given useful information about their virulence Repeated titrations reveal differences between log ID_{50} and LD_{50}, and these may be used to calculate a pathogenicity index As the lethality titer approaches equivalence with the infectivity titer, the index approaches 0 A low index indicates high mouse pathogenicity, and a high index (i e , 4) indicates an infectivity 10,000 times greater than the lethality In general, isolates from severe human infections, parrots, parakeets and turkeys involved in human outbreaks have yielded a low index Strains from pigeons and ducks and from sporadic infections in turkeys have yielded high indices

Death ensues in 2 to 6 days for the highly virulent and in 8 to 15 days for the low virulent strains, some mice recover In animals infected orally or subcutaneously the course is always protracted Latent infections have persisted for 10 to 12 months. The virus has been isolated from mice treated with antimicrobial drugs as long as 277 days after inoculation (Hurst et al , 1953) At necropsy 2 to 4 days after intraperitoneal inoculation the spleen and the liver look normal, but the abdominal viscera are covered by a thin, sticky exudate consisting of endothelial cells

and leukocytes usually packed with viral bodies There are no lung lesions At necropsy 5 to 10 days after intraperitoneal inoculation the abdominal cavity is filled with a stringy, turbid, fibrinous exudate, rich in viral bodies, the enlarged liver and spleen are coated with a thick layer of fibrin, which is easily peeled off Regions of necrosis varying in size and number are present along the margins of the liver

Psittacosis, ornithosis, human pneumonitis, meningopneumonitis, opossum B, hamster pne[illegible]

tion of the lung. Discrete foci of pneumonia are manifested as limiting infective dilutions of virus are approached, the areas are gray, almost translucent, 1 to 3 mm. in diameter After they have been adapted to survival in the mouse, other representatives of the psittacosis group induce fatal pneumonitis. Except for murine pneumonitis, enzootic abortion of ewes, bovine enteric and encephalomyelitis viruses, which cause no symptoms, intracerebral injection of other members of the group causes irritability, ataxia, convulsive seizures and death within 3 to 6 days The meninges are moist and deeply injected Microscopically, the meningo-encephalitis is characterized by an exudate of polymorphonuclear and mononuclear cells, rich in elementary bodies, extending along the blood vessels into the brain This route of artificial infection is useful to increase the amount of virus and to demonstrate quickly the specific viral elements, provided that the material derived from avian sources is free from contaminants and is relatively rich in virus

In guinea pigs most strains produce only a prolonged febrile illness, if that, when inoculated intraperitoneally. But the highly toxic Louisiana pneumonitis, egret, turkey and mammalian (bovine enteritis and encephalomyelitis and pneumonitis of goats and sheep) viruses are highly virulent. The animals succumb to large amounts of virus within 6 to 10 days after a febrile course of 3 or 4 days with temperatures as high as 41 4° C., visible illness, weakness and progressive emaciation At autopsy enlargement of the spleen is usual; a mucoid, viscous, stringy exudate covers the organs, and in a few animals pulmonary consolidation involves one or all lobes of the lungs.

Some strains produce fatal meningo-encephalitis in rabbits infected by the intracerebral route; occasionally, extensive pneumonic consolidations are produced by intratracheal injection Infection of the rabbit's eye has produced a violent reaction. Pocket gophers are susceptible to subcutaneous infection Cotton rats are highly susceptible to the Louisiana virus by the intraperitoneal, intranasal or intracerebral route Wild and white laboratory rats and deer mice are not very susceptible. Syrian hamsters and squirrels (*Citellus beecheyi*) may be fatally infected by the intranasal or intracranial route. *Macacus rhesus* monkeys may be infected by the intratracheal or intracerebral route, psittacosis and highly toxic turkey ornithosis strains produce typical pulmonary lesions or meningo-encephalitis

Parakeets (*Melopsittacus undulatus*), or lovebirds, from aviaries free from psittacosis are susceptible to intramuscular, intranasal and intracerebral infection Immature birds readily contract the infection by exposure to sick birds shedding the virus in droppings When death occurs during the acute stage, gross pathologic findings are a semipurulent coating over the air sac and the inner lining of the sternum, exudate in the pericardial sac, a large liver occasionally studded with areas of necrosis or infarction surrounded by hemorrhagic zones, a large spleen sometimes spotted with necrotic areas, and large, soft kidneys; only rarely are lesions demonstrable in the lungs

With the exception of the meningopneumonitis virus, none of the mammalian viruses is pathogenic for ricebirds, parakeets or pigeons by any route of inoculation, even in very large doses

At least 57 species of the parrot family act as spontaneous hosts to psittacosis (Meyer, 1952). Among 14 different species of the finch family, Java ricebirds (*Munia oryzivora*), canaries (*Serinus canaria*), Poephila, Cyanospiza finches and Java sparrows (*Padda oryzivora*) contract psittacosis when exposed to infected parrots or parakeets.

Domestic fowl (*Gallus gallus*) are susceptible by injection or by exposure; many contract latent infections, and ordinarily few

succumb Pigeons (*Columba livia*) throughout the world are infected; doves (*Streptopelia risoria*), bleeding-heart doves (*Gallicolumba luzonica* and *cruenta*) and Goura pigeons (*Goura cristata* Pallas) (Andrianne, 1953), occasionally infected, are reservoir hosts (Davis, 1955). Pigeons of any breed that have been proved to be free from infection by repeated serologic tests are readily infected by the intramuscular or intracerebral route Intramuscular injections are commonly fatal to doves; intracerebral injections are required to accomplish this result in pigeons Intracranial inoculation of parrot or parakeet psittacosis viruses rarely produces fatal meningo-encephalitis in pigeons, but ornithosis viruses from poultry invariably do so, even in birds with complement-fixing antibodies in their serum

Ducks (*Anas platyrhynchos*) have succumbed to ornithosis during epizootics (Strauss, 1956), or the infection may be clinically inapparent and persist endemically (Korns, 1955). Immature ducks are readily infected by the intramuscular route with any ornithosis virus The disease may end fatally, or after the bird recovers the virus may persist in the liver and the spleen for several weeks, or as long as 1½ months

Indirect evidence that people in the husbandry of pheasant raising may acquire ornithosis has been further supported by isolation of the virus from the spleen of apparently healthy birds (Eddie and Francis, 1942, Ward and Birge, 1952) The pathogenicity of the strains is similar to that of pigeon viruses

Epizootics in commercially raised turkeys have been reported since 1952 from 5 of the United States and British Columbia Undiagnosed flock infections can constitute a serious menace to persons processing anatomically diseased turkeys The viral agent isolated during epizootics resembles the egret and Louisiana strains in its pathogenicity and endotoxin spectrum (Meyer and Eddie, 1953) Clinically inapparent flock infections have yielded isolates of low pathogenicity. The highly virulent strains induce acute, sometimes fatal, infections by intratracheal or aerosol exposure in young turkeys (Pate et al , 1954), isolates of low virulence cause latent infections In Greece serologic surveys have revealed the probability of infection of turkey flocks there (Michail, 1956)

Wild seashore birds, in particular fulmars, petrels, American herring gulls, laughing gull, willet and the mutton bird (Mykytowycz et al , 1955) have been found infected by isolation of the virus Serologic evidence has indicated that other species may be infected (Pollard, 1955) The fulmar and the mutton bird strains have infected man The existence of a reservoir of ornithosis virus in American and nestling snowy egrets has been revealed among birds captured in the coastal areas of Louisiana The virus is pathogenic for mice no matter what route of administration is used (Rubin, 1954)

Etiology

These viruses pass through a relatively complex cycle of development Early studies by Bedson and Bland (1932) working with the psittacosis virus have been extended to the viruses of lymphogranuloma (Rake, 1947), mouse pneumonitis (Karr, 1943), feline pneumonitis (Hamre et al , 1947) and enzootic abortion of ewes (Stamp, 1951) The sequence in the development is fairly uniform After inoculation in mice, embryonated ducks or tissue culture, the elementary bodies disappear The changes that take place in the virus and in the host cell between the disappearance of the elementary body and the appearance of the initial body are incompletely understood This latent period may represent a resting phase, analogous to the lag phase during bacterial growth, in which fully infectious units of virus have penetrated a host cell but have temporarily exhausted the supply of some constituent required for rapid multiplication (Bedson and Gostling, 1954, Weiss, 1955) There is weighty evidence against the view that these viruses multiply by a mechanism similar to that of bacteriophages The early evidence that they divide by binary fission has received strong support through the recent observations of Swain (1955) and Gaylord (1954)

From 8 to 30 hours later, depending on the strain of virus, infection is manifest by the appearance of round bodies, approximately 800 mμ in diameter, called initial bodies Presumably by binary fission these bodies divide, and as they become more and more numerous they become smaller and smaller until the final elementary body stage is reached The

initial body may produce a cluster of granules enveloped in a membrane and a cementing matrix of a density depending on the strain of virus With dense matrix the structures appear homogeneous in stained preparations and are called plaques or morulae. With a light matrix the structures appear granular and are called vesicles. Both types develop into large vesicles that may contain both plaques and granules Tissue cultures reveal no obvious changes until the vesicles are fully developed, when the host cell is suddenly lysed The cytoplasmic plaques or inclusion bodies are true virus colonies made up of elementary bodies The exact nature of the matrix is uncertain Although findings in tissue cultures strongly indicate that it may be a product of the cytoplasm, it may be a secretion of the elementary bodies (Findlay, 1938).

About 30 to 54 hours after the beginning of the cycle, vesicles, morulae and possibly plaques break up, and lysis of the host cell releases several hundred to a few thousand elementary bodies Virus titers parallel the number of elementary bodies. Each elementary body can initiate new cycles in susceptible cells

Variations in this cycle are related to the strain of virus and to the susceptibility of living cells Virus colonies composed of particles invariably contain the virus antigen within the cytoplasm of infected cells (Buckley et al, 1955) During the early growth of mouse and feline pneumonitis virus in the mouse lung, extracellular growth in the alveolus has been seen Vesicles containing granules protrude into the alveolus, apparently attached to its wall (Loosli and Ritter, 1948; Weiss, 1949)

The entoderm cell of the yolk sac is susceptible to all agents of the group, and the yolk is a favorable medium for their survival (Weiss, 1955). Successful propagation of the virus in the chick embryo infected by the chorio-allantoic, the amniotic, the allantoic and the yolk-sac methods provides highly infective suspensions needed in serologic and

chick embryos, these viruses may vary considerably; feline and murine pneumonitis virus grow poorly. Psittacosis and ornithosis strains are readily grown in HeLa or monkey kidney cell tissue cultures, but the yield is low. They exhibit no special affinity for cells of the chick embryo and multiply in the most readily available cells. Infected allantoic fluid, yolk sac or mouse lung are useful for separating the elementary bodies from tissue components by procedures that include the use of proteolytic enzymes, surface active agents, absorption of cell components by specific antiserum or to celite and cycles of high and low-speed centrifugation (Zahler and Moulder, 1953) or simply by differential centrifugation (Gogolak and Ross, 1955). Purified preparations of meningopneumonitis virus were also obtained by dialysis against distilled water (Crocker, 1954). Fairly purified preparations have been obtained and are useful in a diversity of studies, but these procedures bring about considerable inactivation By means of ingenious counting methods it has been learned that 200 to 1,000 elementary bodies of meningopneumonitis (Crocker, 1954) or 20 to 100 feline pneumonitis particles (Moulder and Weiss, 1951) are required to infect chick embryos by the yolk-sac route A linear relationship between the feline pneumonitis concentration and the number of cells infected in tissue culture indicates that one single particle suffices to initiate infection (Weiss, 1955)

The electron microscope shows the size of elementary bodies to range from 350 mμ, meningopneumonitis and bovine enteritis viruses being the smallest, to 500 mμ, feline pneumonitis virus being the largest, various avian viruses and murine pneumonitis virus occupy intermediate positions (Kurotchkin et al., 1947). Filtration through graded collodion membranes and the sedimentation constant give less accurate measurements because the elementary bodies are too large for accurate ultracentrifugal studies The air-dried specimens present a uniform morphologic feature: there are a central zone of high electron scattering power with a wrinkled convoluted surface and a less dense peripheral zone. This outer component in the hydrated particle seems to be a membrane with osmotic properties (Heinmetz and Golub, 1948).

These viruses have an affinity for basic dyes, particularly basic fuchsin (Macchiavello method) and methylene blue (Castaneda method). They do not retain gentian violet

after decolorization with 95 per cent ethanol as well as the rickettsiae do Elementary bodies and larger forms are Feulgen positive

According to phosphorus determinations, the feline pneumonitis virus is of the same chemical complexity as rickettsiae and bacteria; both pentose and desoxypentose nucleic acids in the approximate ratio of 2 5 1 have been identified (Zahler and Moulder, 1953, Moulder, 1954).

The susceptibility of viruses of the psittacosis-lymphogranuloma group to common physical and chemical inactivation is relatively high. They are among the less stable viruses under ordinary laboratory conditions The agent of turkey ornithosis in 20 per cent mammalian tissue suspension has been destroyed in less than 5 minutes at 56° C. and in less than 48 hours at 37° C When a portion of the same suspension was stored at −20° C or at dry-ice-chest temperature for 400 days the virus was gradually inactivated until 99 95 per cent had been destroyed Diseased turkeys frozen in processing plants retained the ornithosis agent in a viable state after a little over a year of storage at −20° C or below When preserved in 50 per cent glycerol in buffered saline (pH 7 6) and held 0° ± 4° C heavy suspensions of infective tissue retained their activity for 10 to 20 days These viruses in sputum and human lung tissue rapidly lose potency in glycerol Formalin (0 1%) or phenol (0 5%) inactivate them in 24 to 36 hours, while ether or ethanol at room temperature is destructive within 30 minutes The pH range of stability is narrow, and the rate of inactivation by ultraviolet irradiation is comparable with that of *Escherichia coli* (Moulder and Weiss, 1951)

Studies dealing with the biochemical aspect of virus growth have furnished only a partial answer to the question: How do the host and the virus parasite participate in virus synthesis? The chemical composition of these viruses, their content of both kinds of nucleic acid, as well as lipid and protein-bound phosphorus, suggest a structural complexity compatible with independent enzyme activity This conclusion is supported by their susceptibility to chemotherapeutic agents known to inhibit bacterial enzyme systems (Moulder, 1954) In this connection the studies on growth factors (Hare and Morgan, 1954, Johnson and Morgan, 1956, Morgan, 1954, 1956) promise to reveal pathways of protein metabolism Virus multiplication has been brought to a halt by disturbing enzyme-substrate complexes and mechanism of catalysis, particularly with respect to metabolic cycles in protein synthesis beginning with certain amino acids, such as phenylalanine and tryptophan The present evidence suggests that the psittacosis-lymphogranuloma venereum viruses are active metabolic units

Early studies on the complex antigenic structure of these viruses showed that at least 2 antigens—one, a heat-labile antigen destroyed by temperature above 60° C, the other, able to withstand boiling or even autoclaving at 135° C—are associated with the elementary bodies of psittacosis (Bedson, 1936) Antigenic components associated with the virus particle are released into solution These are reactive in the hemagglutination, the complement-fixation, the dermal sensitivity and probably the toxin-neutralization tests (Weiss, 1955) The heat-stable component, constituting the bulk of antigen in the elementary body, acts in the complement-fixation and the hemagglutination tests It is the group-specific antigen shared by all recognized members of the group The heat-labile component is the strain-specific antigen shared only by some members, and the serologic activity of this antigen is always much the weaker of the two (Monsur and Barwell, 1951) It may be obtained by treating crude antigens with potassium periodate, which destroys the group component, leaving the rather fragile specific antigen. The marked heat stability of the group antigen and its destruction by periodate indicates that a carbohydrate fraction is a corporate member Now that better methods of purifying virus preparations have been developed, antigens have been identified that are responsible for group and specific reactions in complement fixation, virus toxin neutralization and dermal sensitivity Ether-soluble and ether-insoluble group complement-fixation antigens have been secured from ornithosis-infected yolk sacs (Volkert and Moller Christensen, 1955) Extraction of elementary body suspensions with lauryl sulfate has yielded a water-soluble group complement fixation and dermal antigen identified as a protein-carbohydrate-lipid

complex (Benedict and O'Brien, 1956). By centrifugation the complement-fixing antigen was sedimented, leaving in the supernatant fluid the allergenic antigen which elicits specific dermal reactions in infected turkeys (Benedict and McFarland, 1956). Further progress has been made in isolating alkali-soluble and ether-soluble complement-fixing antigens by chemical fractionation of purified psittacosis or feline pneumonitis elementary body suspensions disrupted by sonic vibration (Ross and Gogolak, 1957) The alkali-soluble fraction could be deprived of its group activity with either potassium periodate or cobra venum (lecithinase A), and thus psittacosis and feline pneumonitis species-specific antigens could be obtained Similar treatment of ether-soluble antigens yielded only group serologic activity Chemical analysis of the alkali-soluble fraction suggests that both the group and the specific serologic activity reside in a lecithin nucleoprotein complex Whether lecithin acts as a hapten in the serologic reactions or potentiates the nucleoprotein component is unknown. Allantoic fluid from duck embryos infected with psittacosis, murine or feline pneumonitis and meningopneumonitis viruses agglutinates mouse erythrocytes The serologic reaction is group- rather than strain-specific. The hemagglutinin consists of 2 chemical fractions: a phospholipid and a nucleoprotein. The former contains lecithin, which is not serologically specific but can agglutinate mouse red cells Specific hemagglutination inhibition takes place in the serum of roosters immunized with purified elementary bodies, indicating that hemagglutinins are associated with the virus particle (Hilleman et al, 1951; Gogolak, 1954, Gogolak and Ross, 1955).

Toxins produced in high concentration in the yolk sac of embryonated hen's egg heavily infected with these viruses is variably lethal to mice on intravenous inoculation (Cox, 1953) The toxicity is unstable; the potency declines at 4° C and is lost at 22° to 37° C. and is destroyed by 0 1 per cent formalin, although the antigenicity is retained The toxin is closely associated with the elementary bodies and is sedimented with the virus when centrifugated at 18,000 rpm for 60 minutes or when put through Seitz filters Certain toxins in the dilution of 1:1,000 (e g, that of the highly virulent turkey strains) kill 50 per cent of the mice within the first 16 hours after intravenous injection; later deaths presumably due to infection begin 24 hours after the inoculation. Using the suppressing effect of antibiotics, the death rate in the first period is often reduced as much as 50 per cent, indicating that some strains multiply rapidly enough to enter into the first phase of the diphasic death curves (Manire and Meyer, 1950). If homologous toxin-virus mixtures and antiserum are administered together, deaths due to the toxin do not occur Potent antiserum produced by hyperimmunization of roosters neutralizes both virus and toxin As a rule, from 80 to 90 per cent of the mice survive at least 48 hours During the following 8 days a variable percentage, never exceeding 50 per cent, succumb to infection Not all strains produce 2-phase death curves Some strains are not lethal by the intravenous route; others with very lethal toxins are rapidly lethal; the death curve is then characteristically monophasic The most potent toxin-producing strains are generally, but not always, associated with the viruses recovered from severe to fatal human illnesses, e g, Louisiana, Illinois and turkey viruses. The toxin-producing ability of most avian strains varies, of the mammalian strains only the enzootic abortion of ewes virus produces a potent toxin The toxin neutralization technic is of value in distinguishing the avian from the mammalian strains (Meyer, 1954)

In numerous publications since 1952 Giroud and his associates have called attention to a diversity of syndromes in man and animals which, according to their interpretation of virus isolations and serologic tests, are caused by intracellular agents related to the psittacosis group, but they have placed the responsible organisms in the newly created genus *Neorickettsiae* Using a micro-agglutination and a very sensitive complement-fixation test with antigen prepared from an Argentinian parrot virus (T13) they discovered that serum of man and cattle, sheep, dogs and cats in Africa, Central America, the Far East and Europe reacted with high specificity and in high dilutions not infrequently with their antigen and quite often simultaneously with a rickettsial agent isolated from a case of exanthema (X14) These extensive reports in

general indicate that certain rickettsiae contain antigens related to the psittacosis group antigen, and that infections with these parasites must be considered in the diagnosis of psittacosis when there is no known association with the usually recognized reservoirs

Another interesting antigenic relationship has been discovered—between an ornithosis virus and certain coccoid bacteria related to the anitratum bacteria (Volkert and Matthiesen, 1956). Human ornithosis positive sera tested with the bacterial antigen showed a positive reaction with the same titers as those obtained with the ornithosis antigen The bacterial culture absorbed at least some of the ornithosis antibody Bacteria of the anitratum group are very common in the respiratory tract of human beings If under certain circumstances these bacteria can produce antibodies reacting with ornithosis antigen, an explanation of some of the false positive reactions may be at hand

The persistence of psittacosis parasites in their hosts in a state of well-adjusted parasitism explains the relative immunity to this infection Probably most patients recovering from psittacosis are resistant to reinfection Second attacks have been observed, and some have been reported (Meyer, 1939-40) One patient after recovery continued to shed virus in the sputum 8 years after severe clinical psittacosis (Meyer and Eddie, 1951) Recovery from an inapparent infection with the turkey virus probably gives no protection against a psittacosis virus These observations are compatible with the recognition that members of the group possess species-specific antigens (Hilleman, 1945, Ross and Gogolak, 1957) The immune reaction of the host consists of the production of antibody barriers against spread of the viruses Using a delicate technic, neutralizing antibodies were found in the serum of some human beings who have a history of psittacosis and of primates recovered from psittacosis (Rivers and Schwentker, 1934) The neutralization of viral activity by antiserum is valuable in identifying many members of the group (Hilleman, 1945, Meyer, 1954) After recovery from psittacosis or during active immunization with active or inactivated virus, specific antibodies are demonstrable in the serum of mammals, including man, and birds. Particularly useful are the complement-fixation and the precipitation tests and the agglutination test (Lazarus and Meyer, 1939) The hemagglutination-inhibition and the conglutinating complement-absorption test (Hilleman et al, 1951) and the indirect complement-fixation test have been used to study the infection in birds (Karrer et al, 1950) The role of opsonins in the serum of recovered animals and the destructive action exerted by monocytes derived from immune mice have been studied in preliminary experiments (Meyer, 1941a; Yanamura and Meyer, 1942) Persistence of serum antibodies at high titer for months after recovery is usually associated with latency Simultaneously, a state of hypersensitiveness is demonstrable by intradermal injection of suitable specific antigens either prepared by extraction of suspensions of psittacosis virus with hydrochloric acid (Bedson et al, 1949) or by special purification (Benedict and McFarland, 1956)

Hyperimmunization with formalized psittacosis vaccine did not completely prevent multiplication of the virus in mice (Bedson 1938) Immunity was effective when immunizing and challenging doses were given by the intraperitoneal route (Yanamura and Meyer 1942), but mice hyperimmunized by the intraperitoneal route with killed vaccines resisted at best 10 intracerebral or intranasal LD_{50} (Wagner et al, 1946) Considerable immunity may be conferred by formalized noninfective vaccines in parakeets and ricebirds (Meyer et al, 1942), and in ewes, formalin-inactivated enzootic abortion virus has eliminated abortion if the vaccine, prepared from infected ovine fetal membrane tissues, was incorporated in an adjuvant (McEwen and Foggie, 1956) A more effective immunity has been obtained with living virus vaccines In guinea pigs inoculated intradermally with virulent psittacosis or murine pneumonitis strains, resistance to intratracheal challenge was developed with 40 respiration LD_{50} doses (Wagner and Victor, 1953) These results are similar to those of an earlier experiment of Rivers and Schwentker (1934) in which monkeys were immunized against intratracheal inoculation by intramuscular injection of small doses of virulent psittacosis virus. Cross-immunity studies indicate that the San Francisco, Illinois and Louisiana strains are rela-

tively homotypic, strains of psittacine origin are less specific, and the ornithosis viruses have broad antigenic structures (Wagner et al, 1949) The method offers too many obstacles to bring strain relationship into categorical positions

Diagnosis

The clinical picture, the results of physical examination and the roentgenogram give useful but not pathognomic information. The symptoms and the history of contact with birds, wild or domestic, living or dead, justify bias in favor of the diagnosis Unfortunately, these must suffice to guide the physician in the care of his patient, because the results of the only certain tests are often not available until the peak of the acute phase has passed Familiarity with the illness in all of its forms—from the mild, atypical, ambulatory, influenzalike disease to the toxic fulminating pneumonitis—should lead the physician to ask the questions necessary to link the patient to the source of his infection In the individual case the success of antimicrobial therapy might seem to obviate the need for diagnosis of this infection altogether But epidemicity is one of its disturbing characteristics, spread from person to person is not unheard of, and the mortality rate is still not negligible The source of one infection may be the source of one or many more

Proof that the illness, not necessarily respiratory, is psittacosis rests on fulfilling the following criteria (1) The viral agent must be isolated during the acute or chronic phase or at autopsy. (2) Complement-fixing, agglutinating or neutralizing antibodies must be found during convalescence or, more certain, the titer of these antibodies must rise during recovery (3) The pathologic, anatomic and experimental examinations of diseased or dead birds in the patient's environment must reveal this agent The probability that these criteria will be met is declining

For primary isolation, blood, sputum, throat washings, vomitus or emulsions of organs are injected intraperitoneally or intranasally into mice, or when free from bacterial contamination, into embryonated eggs (cf Meyer and Eddie, 1956a). These specimens must be taken before treatment Parakeet, parrot and some turkey viruses produce fatal infections in mice by the intraperitoneal route Those from pigeons, ducks and chickens must be enriched through passage first by the intraperitoneal route and then by the intranasal or the intracerebral route for microscopic examination

Between 1931 and 1944 virus was isolated from the specimens of 55 (24%) of 227 clinically and epidemiologically diagnosed cases of psittacosis. Between 1945 and 1953 the proportion of isolations dropped to 23 (4 7%) in 481 specimens (Meyer, 1955) Probably this can be accounted for by treatment with antimicrobial drugs before the collection of the necessary specimens This is regrettable, because the study of isolated strains provides the epidemiologist and the public health worker with knowledge required.

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Complement-fixing antibodies against these viruses appear in exceptional instances before the 12th day after onset Average maximum titers of 1 64 to 1:256 and in rare instances above 1.512 may be reached by the 20th to the 30th days The reaction is subject to wide individual variations The titer may decline within the first 4 months, or it may persist or decline over a period of several years Treatment may partially block or delay antibody

formation. Anamnestic titers rise in other infections, for example in brucellosis or Q fever. If suspicions persist despite the absence of a demonstrable rise in the convalescent specimen, a 3rd or even a 4th should be examined. In cases in which treatment has been vigorous and the test made repeatedly, antibodies have not yet appeared even after 3 months (Meyer and Eddie, 1956a). Anamnestic reactions usually have disappeared by the 30th to the 40th days, while in true psittacosis the titer will usually rise as recovery proceeds. Antibody due to inapparent or latent infection with lymphogranuloma-venereum virus may be a serious cause of confusion. For example, if Caucasian workers in a poultry-processing plant have no antibody to psittacosis, the titers in the Negro workers is more likely to be due to lymphogranuloma venereum, the incidence of overt and latent infection is decidedly higher in the latter than the former (Mandel and Jordan, 1952). Persons who are exposed to psittacosis or ornithosis virus—aviary owners, pet-shop employees, workers in poultry plants—may show clinically insignificant complement-fixing antibodies in their serum in titers up to 1:32.

Despite the often-repeated admonition that a meticulous environmental history is essential, in over 25 per cent of the officially listed cases the source of infection has not been established. Isolation of virus is the most conclusive method in individual birds, but this time-consuming method need not be used to detect flock infections. The complement-fixation test is relatively simple and rapid (Kissling et al, 1956). Chicken, duck and turkey sera are seldom positive in this test because they block the usual reaction. The indirect or inhibition complement-fixation test, on the other hand, has yielded valuable information about the extent of ornithosis in poultry flocks (Karrer et al, 1950, Meyer, 1952). For parakeets it is better to use both tests (Kissling et al, 1956). Using detergent-extracted antigens, the direct test has been used for diagnosis of ornithosis in turkeys (Benedict and McFarland, 1956).

Work is being done on the development of other tests that may be of clinical use—the rapid agglutination test, similar to the Brucella plate test (Mason, 1957), a capillary tube technic similar to that for *Rickettsia burneti* (Mason, 1957) and a skin test (Benedict et al, 1955, 1956). The hemagglutinin-inhibition and the conglutinating complement-absorption tests (Hilleman et al, 1951) are mainly research tools. The technics used to identify and classify isolates are: (1) pathogenicity and toxicity tests in different species of animals, (2) cross-immunity tests, (3) complement-fixation tests with strain-specific antigens and serum and (4) toxin and virus infectivity neutralization tests for demonstration of specific serotypes (Meyer, 1955).

Treatment

Drug therapy has scored noteworthy successes. The early studies are largely of interest in respect to the dynamics of virus infection, particularly in delaying or stopping virus multiplication without complete eradication, thereby allowing development of a carrier state. Since 1950 interest has been centered on tetracycline compounds, because these have been quite effective in treatment, given proper conditions to terminate the infection. In chick embryos chlortetracycline is 5 times [redacted] effective as chloramphenicol on a molecular basis, and 3 times as effective on a weight basis. Tetracycline and oxytetracycline are superior to chlortetracycline in suppressing feline pneumonitis (Katz, 1956), Oxamycin is inferior to chlortetracycline but does lengthen the survival time of embryos infected with feline pneumonitis virus (Cuckler et al, 1955). Erythromycin, Xerosin, nitroacridin 3582 are somewhat active. Chlortetracycline is the preferred drug for human psittacosis caused by psittacine, ornithine and mammalian strains (Fitz et al, 1955). Prompt symptomatic improvement is commonly assured, provided that treatment is begun early with large enough doses and is continued long enough. Inadequate therapy may be followed by relapse. Broad-spectrum antimicrobial drugs did not control pigeon ornithosis virus infection in infants (Berman et al, 1955). It may be that in these cases the drugs, being merely virustatic, received little or no support from immunogenesis, which failed in the malnourished infants suffering from gastro-enteritis and dehydration.

Early studies on treatment of avian psittacosis and pigeon ornithosis were discouraging, but in later studies daily parenteral or oral

tively homotypic, strains of psittacine origin are less specific, and the ornithosis viruses have broad antigenic structures (Wagner et al., 1949) The method offers too many obstacles to bring strain relationship into categorical positions.

Diagnosis

The clinical picture, the results of physical examination and the roentgenogram give useful but not pathognomic information The symptoms and the history of contact with birds, wild or domestic, living or dead, justify bias in favor of the diagnosis. Unfortunately, these must suffice to guide the physician in the care of his patient, because the results of the only certain tests are often not available until the peak of the acute phase has passed Familiarity with the illness in all of its forms—from the mild, atypical, ambulatory, influenzalike disease to the toxic fulminating pneumonitis—should lead the physician to ask the questions necessary to link the patient to the source of his infection. In the individual case the success of antimicrobial therapy might seem to obviate the need for diagnosis of this infection altogether But epidemicity is one of its disturbing characteristics, spread from person to person is not unheard of, and the mortality rate is still not negligible The source of one infection may be the source of one or many more

Proof that the illness, not necessarily respiratory, is psittacosis rests on fulfilling the following criteria· (1) The viral agent must be isolated during the acute or chronic phase or at autopsy (2) Complement-fixing, agglutinating or neutralizing antibodies must be found during convalescence or, more certain, the titer of these antibodies must rise during recovery (3) The pathologic, anatomic and experimental examinations of diseased or dead birds in the patient's environment must reveal this agent. The probability that these criteria will be met is declining

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ington, bringing the total for 1956 to 134 cases, and 27 from British Columbia in 1957. It has been estimated unofficially that 486 cases (12 deaths) have been contracted through exposure to turkeys in these places. Serologic surveys have indicated that flocks carry latent infections in California, Michigan, Minnesota, Massachusetts, Ohio and Arizona. Samples of normal and moderately enlarged spleens from 2 flocks in California and Michigan have yielded isolates of low or moderately high virulence. These have caused no known human infections. In contrast, the highly virulent strains isolated from turkey flocks during epizootics caused human attack rates as high as 48.2 per cent in the processors. The sources of infection and mode of spread, in fact the whole ecology of turkey ornithosis, are not known.

Obviously, both psittacine and extrapsittacine birds serve as reservoirs and transmit the virus to man. In 1954 nearly 60 per cent of the 563 cases officially reported were attributed to sources other than psittacine birds. In fact, of the 1,610 cases on record for 1952 to 1956 (first 9 months), the source of infection was learned in 808. 448 (55%) were of psittacine and 360 (45%) of extrapsittacine origin, mostly turkeys. Human infections directly traceable to mammals are either unrecognized or occur infrequently. Only 2 laboratory infections one proved through isolation of enzootic abortion virus from the sputum (Barwell, 1955) and the other by isolation of bovine encephalomyelitis virus from the blood (Meyer and Eddie, 1956b), furnish evidence that the mammalian viruses can infect man. There is additional evidence in the antibody response of veterinary personnel in stockyards (Enright and Sadler, 1954) and sheepherders (Gerloff and Lackman, 1954) in the United States. An infection among natives in the Belgian Congo has been attributed to the related *Neorickettsia* by Giroud and Jadin (1954), who have suggested that these outbreaks may have originated from cattle, sheep or goats.

The epidemiologist must carefully investigate every known and even unknown avian and mammalian source, rather than rely solely on the possibility of human spreaders of the virus.

With removal of virtually all restrictions on the movement of psittacine birds in 1951, mainly in the United States, the exploitation of a nation-wide market for domestically reared and illegally imported parakeets has increased the annually reported cases from 135 in 1952 to 563 in 1954 and 508 in 1956. The reported case rate per million population for 1952 was 2.3, for 1954, 4.3, while the number of pet birds in the United States rose from about 3 million in 1940 to around 15 million in 1953. In other words, the psittacine population increased many more times than the reported case rate.

In the past, single cases undoubtedly escaped detection; old reports deal principally with house epidemics which established the pattern now so well known of the psittacosis epidemic. The great epidemics of the past occurred during the winter probably because of prolonged exposure to infected avian pets in closed rooms of a winter household. Severe infection on the Faeroe Islands and among pigeon fanciers the world over is not uncommon in midsummer and early fall. It now seems probable that it can be contracted throughout the year. Its higher incidence among people of middle age or older has been stressed, but the evidence is open to question. These people may be more likely to acquire birds as pets and to spend more time in closed households. Infants and children under 5 years of age have contracted fatal infection (Berman et al., 1955), and severe illness has been observed in the age group 10 to 20 years (Strobel, 1954). The disease has a greater incidence among women than men. The fairly constant ratio of 2:1 may be ascribed to their activities as parakeet breeders or bird-lovers. Men and women are probably equally susceptible. In processing plants under identical conditions of exposure the attack rates are nearly identical. The higher attack rate in non-Caucasians in processing plants reflects more the nature of the work—killing, defeathering and eviscerating—than susceptibility. Psittacosis is a fairly common occupational infection among persons engaged in raising or trading in parakeets or pigeons and in transporting parakeets (Seibert et al., 1956).

The ways the infection may be contracted, in order of importance, are by inhalation, possibly by the gastro-intestinal tract and through bite wounds. The ease with which inhalation of virus induces pneumonic lesions in susceptible mammals points directly to the respiratory tract as the principal entry for the virus. Handling of sick or dead birds or contact with feathers soiled with infective discharges or excreta or with droppings from latently infected flocks—all capable of creating virus-carrying aerosols—are fully proved modes of infection. The clinical nature of the

therapy with 30 mg per Kg of tetracycline compounds has improved the health of individual parakeets, reduced mortality, prevented epizootics and reduced the hazard of infection in aviary personnel. It does not always eliminate the carrier state in every bird, but if treatment of aviaries is repeated at regular intervals, ultimately the infection may be eradicated (Meyer et al., 1958). Attempts to eradicate ornithosis from pigeon flocks remain disappointing Prolonged treatment with large doses of tetracycline compounds is required to eliminate the carrier stage in parrots During epizootics of ornithosis in turkeys treated with pharmacologically inadequate doses of tetracycline compounds (400 Gm. per ton of feed) for 15 to 30 days the mortality was arrested and the birds visibly improved. But virus persisting in the anatomic lesions and the viscera caused infection in the personnel of processing plants handling the treated birds

The action of these compounds in vitro and in vivo is suppression of multiplication, in particular division of initial bodies (Weiss, 1955) One assumption is that these compounds interfere with some metabolic process essential for reproduction (Moulder, 1954) In addition to their susceptibility to chemotherapeutic agents, one of the characteristics of these viruses is their ability to develop drug-resistant mutants in egg and mouse passage The development of drug resistance in viruses that tend to chronicity may seriously impair the programs designed to eradicate psittacosis from poultry or parakeet stocks by treatment (Gordon et al, 1957)

Epidemiology

The avian sources of infection in man have changed since the outbreaks of 1929 and 1930 Of great significance was the discovery that in the United States and Germany visibly healthy relatives of the parrots, the parakeets, were prolific disseminators of psittacosis virus. For a time after 1932 nearly all reported cases in man were derived from psittacine birds Enzootic and occasionally epizootic psittacosis was proved in aviaries and pet shops in the United States and Europe [illegible] et al., 1956) and from these res- [illegible]

supports the belief that [illegible]

[illegible] parrots, parakeets and [illegible]

ological gardens have been high at times either because the birds had not been immunized by nest infection or because relapses were brought about by low temperature, crowding in dirty cages and improper feeding Shortly after fulmars or petrels were the sources of human illness and death on the Faeroe Islands, in the United States birds other than psittacine were found to be spreading the infection. Then it was learned that pigeons are potential sources, and according to reports from nearly every country in the world where studies have been instituted ornithosis is widespread among wild and domestic pigeons (Mohr, 1954). Squabs with extensive lesions may infect the person who dresses them An increasing literature from Holland, England, France, Italy, Germany, Israel, Mexico and Australia attests to the widespread symptomatic and more often inapparent ornithosis infection in pigeons (Weyer and Lippelt, 1956; Kemmerer et al., 1956) Serologic evidence of ornithosis in ducks in Michigan was subsequently supported by isolation of the virus both from healthy and from diseased ducklings from that state (Meyer and Eddie, 1952) and from ducks on farms in California and Long Island Histories of mild cases of atypical pneumonia among duck handlers were identified as psittacosis in Long Island and Virginia (Andrews, 1957). Serious economic losses among ducklings on farms and more than 100 human infections among women plucking ducks have been reported from Czechoslovakia since 1949 (Konrád and Strauss, 1955).

Chickens (Karrer et al, 1950) and pheasants (Ward and Birge, 1952, Ward et al, 1954) have been incriminated and proved to be infected, though few cases of transmission to man have been reported.

Reports on outbreaks among turkey-processors in Texas (Irons et al, 1955; Irons et al, 1956) call attention to a suspected episode as early as 1938. Since 1948, 7 authentic outbreaks among turkey-dressers have been discovered. During 1954 an outbreak was reported from New Jersey, and another involving husbandrymen, processors and even renderers (86 cases in all) occurred in Oregon in 1956 Thirty-four cases (1 death) followed the dressing and the cleaning of turkeys in 2 plants in Texas; 10 cases have been reported from Wisconsin, 4 from Wash-

History

John Hunter in 1786 described inguinal buboes in the male, and in 1833 Wallace described them and their accompanying constitutional symptoms in greater detail Desruelles gave an excellent account of 2 cases of vulvar hypertrophy following the involvement of the inguinal lymph nodes Hugier is credited with giving the name "esthiomene" to the characteristic induration and discoloration that involves the affected parts Multiple abscesses in inguinal adenitis were originally described by Velpeau, and Nélaton and Tanton and Pigeon recognized them to be the nontuberculous manifestations of an unknown disease (Koteen, 1945). Suppurative inguinal adenitis was described as climatic bubo and attributed to plague, malaria and fatigue, until Rost in 1912 realized that it is a venereal infection Klotz first described the disease in the United States, calling it strumosen Bubonen, and was the first to note the penile lesions, Durand, Nicolas and Favre, 1913, brought these manifestations into one disease entity, subacute inguinal lymphogranulomatosis, transmitted sexually, and they conclusively differentiated it from syphilis and tuberculosis But because of its close resemblance to Hodgkin's disease they sanctioned the designation lymphogranuloma inguinale Phylactos, a pupil of Favre, called it the fourth venereal disease, but emphasized that its characteristics were identical with those of climatic bubo

For a time there appeared to be no correlation between lymphogranuloma venereum and the seemingly independent genital diseases observed by gynecologists and surgeons for nearly 100 years—troublesome ulceration and elephantiasis of the female pudenda—and inflammatory stricture of the rectum Though lymphogranuloma venereum was often associated with venereal disease, its origin was clarified only with the development of the Frei test, and within a few years a bacteria-free meningitis was produced in rhesus monkeys by intracerebral inoculation of pus from a bubo (Hellerstrom and Wassen, 1930)

With introduction of the mouse as a suitable laboratory animal for study of this disease and the finding of virucidal antibodies by Levaditi et al, 1932, began the investigation of lymphogranuloma venereum as a virus infection Gamma, 1924, described large bodies in the cytoplasm of cells from infected lymph nodes, but their nature remained uncertain The small granules reported by Gay Prieto, 1927-28, and the granulocorpuscles of Miyagawa et al (1935) were recognized by Findlay (1938) and by Rake and Jones (1942) as elementary bodies derived from larger initial bodies in an interesting developmental cycle Lymphogranuloma venereum virus was among the first in the group to provide evidence of a thermostable endotoxin and to be found sensitive to sulfonamides

Clinical Picture

Usually within a few days after venereal exposure the initial lesion develops on the glans and the prepuce of the penis, the posterior aspect of the labia, the vaginal walls, the cervix, within the urethra or in the region of the anus This vesicle, also known as the herpetiform lesion of Cole, 1933, bursts, leaving a shallow, grayish ulcer of lymphogranulomatous chancre The clean-cut edges surrounded by a narrow band of reddened skin are not indurated In a rare case the initial lesion is on the tongue or the conjunctiva The vesicle may be painless and therefore overlooked or it may be ignored The infection may terminate at this stage, or it may proceed to the second stage Laboratory infections attest that the disease may have minimal symptoms mild fever fleeting muscle pains and malaise

The second stage is characterized by involvement of the regional lymph nodes, usually in the groin, rarely in the neck, the axilla or the oculoglandular region Pain in the groin and enlargement of the nodes may be the only symptoms When the primary lesion is intra-urethral or anorectal, the infection spreads to the pelvic nodes The lymph drainage of the vagina favors involvement of the intrapelvic the perianal and the deep pelvic nodes In the male the buboes form in the inguinal nodes, at first only discrete, slightly tender and movable Later they adhere to the underlying tissues and form a large, single, tender inflammatory mass In most patients in the temperate zone the adenitis is unilateral, in the tropics it is usually bilateral It may resolve spontaneously, but in about half the cases the nodes suppurate The infection may be confined to the lymph nodes, or it may become more generalized

The adenitis may be accompanied by chills,

illness is related to the dose and the virulence of the virus (Gordon, 1957) and to the portal of entry Little is known about the possibility of infection of man by the gastro-intestinal route. Bovine enteritis virus is excreted in the feces of cattle, and when it is fed to calves induces illness, but the relation of this and the psittacosis and the ornithosis viruses, also excreted, to human disease is unexplored. It seems possible that psittacosis virus might be swallowed after hand-to-mouth transmission from the environment of an infected bird. As far as is known, human beings have not contracted ornithosis by ingestion of infected poultry. When concentrated highly infectious suspensions of the turkey ornithosis agent

man is greatly diminished because ordinarily poultry ■ cooked thoroughly enough to destroy most, if not all, the virus particles.

Infections in laboratory personnel and animal caretakers fully attest to the extreme contagiousness of the disease, which demands utmost caution in the handling of the diverse specimens suspected of carrying the virus *The infection may also spread from person to* person (Berman et al, 1955, Babudieri and Cerri, 1956). At least 23 reports, involving 30 nurses, are known The 1943 epidemic of severe pneumonitis in the bayou region of Louisiana, with a toll of 8 deaths in 19 recognized infections among nursing attendants, emphasizes the importance of direct contact in human-to-human transmission. In one instance a patient known to be a psittacosis carrier for 8 years did not transmit the infection (Meyer and Eddie, 1951)

The case fatality rate for the outbreak years 1929 and 1930 was nearly 20 per cent, *in some outbreaks 40 to 100 per cent, but* with the recognition of more mild infections the rate was about 10 per cent Then with the introduction of antimicrobial drugs it has fallen to 5 per cent in the United States and to 3.5 per cent (86 cases) in Germany (Haussman et al., 1956) It could be kept even lower if early diagnosis and proper treatment were instituted. Most of the patients who died were 40 to 60 years old

Control Measures

Psittacosis is a minor health problem.

parakeets during shipment from mass collecting centers to pet shops and finally the consumers to warrant the organization of preventive programs. With the aid of chemotherapy, immature parakeets could be mass produced, freed from infection, distributed and sold through pet shops Suppression of this infection chain would greatly reduce the number of human infections acquired in homes Educational campaigns must continuously warn the public and physicians to understand the danger from contact with apparently healthy birds Prohibition of taking young fulmars onto the Faeroe Islands has terminated the yearly outbreaks of severe pneumonia The incompletenes of ecologic information on ornithosis in turkey flocks makes it exceedingly difficult to plan and to carry out control measures Chemotherapy with tetracycline drugs has failed to sterilize the virus-laden visceral lesions and to prevent the occupational hazards among workers in processing plants and the marketing of diseased turkeys (Meyer, 1955). In view of the gratifying results in epizootic abortion of ewes through immunization with inactivated virus in adjuvants the prophylactic possibilities of vaccines should be re-explored by the poultry industry until methods have been developed to create and maintain flocks free from infection. Immunization of human volunteers against psittacosis has been carried out with dilutions of active virus subcutaneously. How effective such or improved procedures might be in control of the disease in man is not known

LYMPHOGRANULOMA VENEREUM

(Synonyms: Climatic bubo, tropical bubo, venereal bubo, fifth venereal disease, lympho-

Introduction

This disease is manifested by constitutional symptoms and by acute and chronic tissue changes in the inguinal and the recto-anal regions The virus is related to psittacosis virus in that its developmental cycle includes visible large elementary bodies in mononuclear cells

bits accept the infection more readily than older animals Fowl, pigeons, ricebirds and parakeets fail to react to intracerebral injections of highly infectious passage virus

Infection has been initiated by injecting mice intracerebrally with monkey-passage virus or with human material Of 26 strains transmitted to mice, only 2 were highly virulent; others lost their infectiousness on passage The 2 virulent strains produced symptoms of meningitis in 2 to 4 days. Many strains, when injected intracerebrally, induce, after an incubation time of 7 to 14 days, muscular in-co-ordination, paresis and weakness, with a mortality rate of 12 to 39 per cent In such mice the histologic findings are those of leptomeningitis, and impression preparations from the meninges furnish excellent material for study of the large and the small viral elements Intranasal administration of virus incites a pneumonic process that may be fatal between 4 and 6 days, it is characterized by desquamative alveolitis, nodular inflammation around the capillaries and the lymphatics and the formation of virus elementary bodies When the virus is injected intraperitoneally it localizes in the brain if starch is injected intracerebrally. It may be successfully passaged through the testes of mice

To produce models of the human disease, monkeys have been infected by the intraperitoneal, intrapreputial, intracutaneous, intrapulmonary and intra-ocular routes, and in the tissues of the intestine, the rectum and lymph nodes Typical inflammation develops locally, and the virus was readily demonstrable in the enlarged regional lymph nodes Neither the intravenous nor the intraneural route will establish infection in this animal.

Etiology

The infective agent is a large elementary body virus that passes through its development in the cytoplasm of reticuloendothelial cells Elementary bodies may be demonstrated in cells from human lesions, and they are transferable to experimental animals. They are agglutinated by specific antiserum and serve as specific antigens in the complement-fixation and intradermal tests Observations on the growth cycle of this virus have been correlated in a detailed study by Rake and Jones (1942), who investigated its multiplication in the yolk sac Its structure, developmental forms and staining characteristics place it in the psittacosis group All the basic characters of this group apply, with the exception of minor differences, in particular the species specificity of the antigens

The filterability is not constant By use of gradocol membranes according to Elford's method, the diameter of the infective viral body has been estimated at 120 to 180 mμ Electron micrographs of the elementary bodies propagated in the yolk sacs of developing chick embryos revealed a mean diameter of 438 ± 47 mμ (Kurotchkin et al, 1947) At 37° C the virus remains active from 2 to 4 days, while at 56° C it loses its power to infect within 10 minutes To keep it alive for a year or longer it is best held at −30° to −70° C Ultraviolet radiation renders it noninfective within 30 minutes In 50 per cent neutral glycerol the activity is retained for only a week or two Formalin (0 1%) or phenol (0 5%) inactivates it in 24 to 48 hours Ten per cent ether at room temperature inactivates it in yolk-sac suspensions within 30 minutes This virus can be cultivated in tissue cultures By propagation in the embryonated egg it yields highly infective (LD_{50}, 10^{-8}) suspensions useful for biologic and immunologic studies

Rake and Jones (1944) reported isolating a toxic substance readily demonstrable in heavily infected yolk sacs of moribund embryos It kills young mice rapidly after intravenous injection, if the activity is high and the dilutions are made in the allantoic and the amniotic fluids, it occasionally kills after intraperitoneal injection It is labile, readily inactivated at room temperature and by chemicals It produces hemorrhages in the lungs of mice

Yolk-sac suspensions or extracts contain the heat-stable antigen common to all viruses of this group It gives specific complement-fixation reactions in the presence of serum from lymphogranuloma venereum patients Aside from boiling, the reactivity of the yolk-sac suspension is greatly intensified by the addition of phenol, which suggests that the complement-fixing antigen is probably a lecithin nucleoprotein complex Destruction of the group activity with either potassium periodate or lecithinase should produce a

sweats, fever, prostration, loss of weight, anorexia, nausea, vomiting, pains in the thorax and the muscles, stiffness of the neck, headache, epistaxis and bronchitis. Scarlatiniform rashes and those resembling erythema multiforme have been reported If the infection becomes generalized all lymph nodes, the spleen and the liver may enlarge. The virus is frequently disseminated throughout the body; it has been recovered from the circulating blood, the spinal fluid and material obtained by puncture of the spleen while the buboes are present. Moderate secondary anemia and leukopenia are common. When the nodes suppurate there is often leukocytosis with relatively slight mononucleosis The erythrocyte sedimentation rate is usually increased Hyperglobulinemia with elevated total serum proteins and reversal of the albumin-globulin ratio is common. In severe cases this stage may progress to pneumonitis or to meningoencephalitis Conjunctivitis with oculoglandular syndrome has also been recognized.

The tertiary stage presents itself early or late in the infection in well-defined syndromes or in isolated or associated manifestations in different organs or systems. The more easily recognized are esthiomene, urethrogenitoperineal syndrome, elephantiasis of the penis and scrotum, rectal stenosis and plastic induration of the penis The esthiomene frequently mentioned in the literature is a nondestructive elephantiasis of the preputium, the clitoris or the labia minora. It sometimes extends to the labia majora and other soft parts of the vulva and the anus The effect of the infection on pregnancy is evidently minor The specific proctitis usually arises from spread of the infection from perirectal tissues through the rectal wall. Early in this proctitis the patients have little or no pain If it is not treated, the sequelae may be rectal stricture or vaginorectal or vaginovesical fistula Complete obstruction is uncommon, but the chronic process is frequently complicated by perirectal and perianal abscess and fistula Rectal stricture formerly erroneously attributed to injuries, syphilis or other causes is now being recognized as a sequel to lymphogranuloma venereum (Coutts, 1950).

The clinical characteristics are very well described by Favre and Hellerstrom (1954).

Pathologic Picture

There are no characteristic tissue changes in the lymphogranulomatous chancre; the area surrounding the ulcer is infiltrated mostly by plasma cells and histiocytes containing inclusion bodies The reaction in the lymph

same as that in inguinal and pelvic lymph nodes At times the lesions have some characteristics of those seen in tuberculosis and syphilis, but an experienced pathologist can make the distinction The inflammatory process consists of a great outpouring of mononuclear elements, especially plasma cells, a few neutrophils and eosinophils, and proliferation of the macrophages with giant-cell formation. Epithelioid transformation of the macrophages gives rise to peculiar tubercle-like nodules, which undergo necrosis There is, in addition, marked proliferation of fibrous tissue which, as the lesions heal, contracts, producing

tendency

gro may

the frequency of stricture in this race. The cone or dumbbell-shaped, basophilic gamma bodies and the clusters and the chains of smaller azurophilic elementary bodies have been demonstrated in cells of human lesions by Coutts et al, 1942.

Experimental Infection, Host Range

The first experimental transmission of the disease is credited to Hellerström, 1929, who infected a man by the intra-urethral route Using monkey-passage material, Wassén, 1935, infected Frei-negative patients suffering from general paralysis or dementia praecox; the lesions produced were typical of lymphogranuloma venereum

The span of the host range is still not completely known. It was the successful transmission of the virus to monkeys (*Cercopithecus callithrix*) and the mouse that greatly accelerated knowledge of the disease, its etiologic agent and methods of diagnosis These two are the most highly susceptible animals, less susceptible are the guinea pig, rabbit, squirrel, marmot, harvest mouse, rat, cat, dog and sheep Results have been erratic in experimental infection of the less-susceptible animals. Young rats, guinea pigs and rab-

apparently does not intensify the cutaneous sensitization.

The Frei test usually becomes positive 7 to 40 days after onset of the adenitis. The rare false negative Frei reaction is usually due to such factors as the menses, septicemia, fever, tuberculosis or coexistent early syphilis or early chancroid. *If the clinical picture* suggests early infection, the Frei test should be repeated. In a study of 1,265 patients, 243 gave persistently positive reactions, and all but 17 of the 243 gave a history typical of infection or showed clinical manifestations (Connor et al., 1937). The best available clinical and experimental information suggests that the reaction usually becomes positive 1 *to 6 weeks after* the infection begins and probably remains so for the life of the patient (Palmer et al., 1942). The specificity of the test has been doubted because it has been positive in patients without a history or clinical evidence of lymphogranuloma venereum and especially in patients suffering from syphilis (Robinson, 1940). In the clinical cases, the specificity is now considered to be of a high order, and the results closely parallel those of the complement-fixation test, using yolk-sac antigen in both. In viral pneumonia caused by other members of the psittacosis-lymphogranuloma venereum group the intradermal test with *Lygranum* or a similar preparation with a psittacosis virus may be positive. Furthermore, in occasional cases of lymphogranuloma venereum diagnosed on clinical grounds the intradermal test has been strongly positive but the complement-fixation test negative. Specific antigen prepared by acid extraction of elementary bodies give reactions only in patients infected with lymphogranuloma venereum. Successful treatment early in the infection may reverse the cutaneous sensitivity (Koteen, 1945). Under cortisone therapy the allergic reaction may temporarily disappear, while the complement-fixation reaction remains positive.

4. Propagation of the virus in the yolk sac or the lungs furnishes potent antigens for use in the complement-fixation test. Since the heat-stable group antigen is the active component of the reagent, it has been suggested that the enzootic abortion virus, which is safe to handle and grows to a higher titer, be used (Dane, 1955). In Heyman's study (1946), all of 27 patients with early lymphogranuloma venereum, proved by isolation of the agent, had complement-fixation titers of 1:40 or more, and most had titers of 1:160 or higher. The geometric mean titer in 45 symptomatic and asymptomatic persons was 1:80 (Greaves and Taggart, 1953). However, more *important than the height of the titer is the* serologic trend determined by study of acute phase and convalescent serum collected over a period of weeks. A single positive complement-fixation reaction as a rule is not sufficient basis for a serologic diagnosis. The serum of patients who have recovered from psittacosis may give a positive complement-fixation reaction with lymphogranuloma venereum antigens. *Patients suffering from* lymphogranuloma venereum or psittacosis give low-titer complement-fixation reactions with an antigen prepared from conjunctival scrapings of patients suffering from well-developed trachoma. On the other hand, positive complement-fixation tests with serum of trachoma patients in the presence of psittacosis or lymphogranuloma venereum antigen are rare and of low titer (Babudieri et al., 1955). The ability of the serum of lymphogranuloma venereum patients to react with the psittacosis antigen may be removed by absorption of the group antibody with steamed lymphogranuloma venereum virus (Bedson et al., 1949). This somewhat laborious procedure leaves the major part of the antibody intact, the absorbed serum gives a specific complement-fixation reaction with unheated lymphogranuloma venereum virus, but not with the psittacosis virus. The complement-fixation test serves as a useful instrument for screening population groups and for gauging the incidence of lymphogranuloma venereum, it has special utility in epidemiologic studies because it can be performed concomitantly with a serologic examination for syphilis.

It is quite apparent that no relation exists between the titers of the complement-fixation test and the intradermal test in symptomatic or asymptomatic lymphogranuloma. It is reported that in 54 to 62 per cent both reactions are positive (Greaves and Taggart, 1953). Of 1,119 patients in a venereal disease clinic, 206 (18.4%) gave positive Lygranum skin tests, while only 24 (2%) had complement-fixation antibodies in titers high enough

lymphogranuloma venereum species-specific antigen of the same character as the parakeet psittacosis antigen (Ross and Gogolak, 1957). This has not been tried.

An attack of lymphogranuloma venereum probably induces a lasting immunity (Koteen, 1945). An infected person is fully refractory to a cutaneous or intradermal reinfection with material proved to contain the virus; a local reaction similar to that induced by inactive virus (Frei reaction) develops at the site of insertion, but neither the skin nor the regional lymph nodes show signs of infection. Whether or not the immunity survives the disappearance of the virus from the host has not been determined. Prolonged coexistence of infection and immunity is characteristic of this group of agents. Consequently, clinical relapses have been reported and may be anticipated. A fairly large percentage of completely recovered experimental animals have been immune to intranasal or intracerebral reinfection

There is evidence that injection of inactivated preparations leads to the development of immune bodies and resistance to moderate infection There are no records of human immunization with vaccines.

Reports are contradictory regarding the production of neutralizing antibodies Negative results are attributable to the use of faulty technics, such as too short incubation of virus-serum mixtures or use of serum dilutions in the presence of concentrated viral suspensions. Hyperimmunization of chickens (Hilleman, 1945) with yolk-sac suspensions invariably produces potent neutralizing antiserum which has protective, toxin-neutralizing, agglutinative and complement-fixation inhibiting properties. Such serum conveys no cross protection against infection with any other member of the group. The serum of guinea pigs, rabbits, mice or rats immunized with this virus gives strong complement-fixation reactions with the homologous antigen, and frequently an equally marked reaction will occur with heterologous antigens prepared with other members of the group The complement-fixation test indicates a broad antigenic similarity between the members of the group; however, unexplained differences in titer are on record (Smadel et al., 1943).

Diagnosis

Lymphogranuloma venereum must be differentiated from chancroid, bubo due to pyogenic lesions of the lower extremities, tuberculosis of the inguinal lymph nodes, gonorrhea, syphilis, granuloma inguinale, balanitis, plague, tularemia, carcinoma and tuberculosis of the rectum, and ulcerative colitis.

1. Films of pus should be examined for organisms, and cultures should be prepared aerobically and anaerobically, not omitting the rabbit blood technic for Ducrey's bacillus If organisms are not found it is advisable to make special examinations

2. Biopsy material should be collected, and smears of bubo pus or even tissue sections are stained with Giemsa or Noble stain for the demonstration of the elementary bodies Unfortunately, histologic examinations are not commonly made Changes in chancres and lymph nodes are characteristic enough to distinguish them quite accurately from similar lesions of other venereal diseases Material from genital lesions should be subjected to darkfield examination.

3 The intradermal test of Frei (1925) is used extensively as a practical test It consists of intradermal injection of 0.1 ml. of antigen and 0 1 ml of control material. The test is read 48 and 96 hours after injection A raised papule measuring 6×6 mm. or greater indicates a positive reaction if the papule produced by the antigen control is 5×5 mm or smaller. Originally, the Frei antigen was prepared by diluting fresh pus or pus dried from the frozen state from the bubo of a known human infection with 5 times its volume of sterile saline solution Then it was sterilized by heating at 60° C for 2 hours on one day and for 1 hour on the next In antigens prepared from infected monkey or mouse brain, although specifically effective if properly controlled, the virus content was frequently low and reactions doubtful. A satisfactory antigen can be prepared from the yolk sacs of chick embryos moribund or recently dead from infection (Rake et al, 1940). Control material is prepared from the normal yolk sac of 10-day chick embryos The skin test material is known commercially as Lygranum Positive reactions to yolk-sac antigens remain readily visible and palpable for 10 days and longer, and repeated testing

(Luger, 1950). This high incidence is not indicative of any known racial predisposition; several factors in the unhygienic environment probably influence the occurrence of larger numbers of cases. In London, of the 23 patients in a venereal disease clinic with strongly positive complement-fixation reactions, 14 were Caucasian, 9 were Negro. Two per cent of completely asymptomatic patients apparently had latent infection (King et al, 1956). The idea that the infection is very rare in the Nordic countries deserves reconsideration and broader epidemiologic investigations in the light of these findings and the demonstration of positive complement-fixation reactions with antigens made with members of the psittacosis-lymphogranuloma venereum group in persons with no proved contact with avian reservoirs.

Control Measures

Modern plans for combating syphilis and gonorrhea, emphasizing as they do case finding and medical care and control of infected persons, are applicable to lymphogranuloma venereum. The first step is to make the infection reportable. Adequate diagnosis, including the complement-fixation test, must be made available through local health departments. Since serologic examinations are less expensive and more readily carried out, in the routine examination of sexually promiscuous persons the complement-fixation test should be used. The city health department should provide, free of charge, drugs for treatment. Early intensive treatment of clinical and subclinical infections should be assigned to private physicians or special clinics. Public education and the information of physicians of developments in diagnosis and treatment are other important steps.

REFERENCES

(For complete references, see also 2nd edition, Chapter 20.)

Andrews, J. M., 1957, The importance of psittacosis in the United States, J Am Vet M A *130*, 109-116.

Andrianne, V F, 1953, Sur quelques cas fatals d'ornithose chez des pigeons Goura (*Goura cristata*-Pallas). Isolement du virus. Traitement à la terramycine. Ann méd vét *97*, 63-72.

Austin, F J, 1957, Psittacosis, J A M A *164*, 1607.

Babudieri, B, Bietti, G B, and Pannarale, M R, 1955, Ulteriori contributi sull'esistenza di affinità antigeniche fra gli agenti del tracoma e quelli del gruppo linfogranuloma venereo-psittacosi, Rendic Ist super san *18*, 849-853.

Babudieri, B., and Cerri, R., 1956, Osservazione di casi umani di ornitosi a Genova, Rendic Ist super san *19*, 59-66.

Baker, J A., 1944, A virus causing pneumonia in cats and producing elementary bodies, J Exper Med *79*, 159-172.

Barwell, C F, 1955, Laboratory infection of man with virus of enzoötic abortion of ewes, Lancet *2*, 1369-1371.

Barwell, C F., and Bishop, L. W J, 1951, Virus of enzootic abortion in ewes: antigenic relationship with viruses of the psittacosis group, Nature, London *167*, 998.

Bedson, S P, 1936, Observations bearing on the antigenic composition of psittacosis virus, Brit J Exper Path *17*, 109-121.

———, 1938, Study of experimental immunity to the virus of psittacosis in the mouse, with special reference to persistence of infection, Brit J Exper Path *19*, 353-366.

Bedson, S P., Barwell, C F, King, E J, and Bishop, L W J, 1949, The laboratory diagnosis of lymphogranuloma venereum, J Clin Path *2*, 241-249.

Bedson, S P, and Bland, J O W, 1932, A morphological study of psittacosis virus, with the description of a developmental cycle, Brit J Exper Path *13*, 461-466.

Bedson, S P, and Gostling, J V, 1954, A study of the mode of multiplication of psittacosis virus, Brit J Exper Path *35*, 299-308.

Benedict, A A, and McFarland C, 1956, Direct complement-fixation test for diagnosis of ornithosis in turkeys, Proc Soc Exper Biol & Med *92*, 768-771.

———, 1956, Antigenic studies on the psittacosis-lymphogranuloma venereum group of viruses IV Studies on cutaneous hypersensitivity in chickens infected with ornithosis virus J Immunol *77*, 165-171.

Benedict, A A, and O'Brien, E., 1956, Antigenic studies on the psittacosis-lymphogranuloma venereum group of viruses II Characterization of complement-fixing antigens extracted with sodium lauryl sulfate J Immunol *76*, 293-300.

Benedict, A A, O'Brien, E, and McFarland, C, 1956, Antigenic studies on the psittacosis-lymphogranuloma venereum group of viruses III Detection of ornithosis hypersensitivity in experimentally infected chickens Am J Vet Res. *17*, 543-546.

Benedict, A A, Tips, R L, and Eddy, D., 1955, Antigenic studies on the psittacosis-lymphogranuloma group of viruses I Acid-soluble complement-fixing and skin test antigens Texas Rep Biol & Med *13*, 206-212.

Berman S et al, 1955, Ornithosis in infancy, Pediatrics *15*, 752-760.

Binford, C H, and Hauser, G H, 1944, An epidemic of a severe pneumonitis in the bayou region of Louisiana III Pathological observations. Report of autopsy on two cases with brief comparative note on psittacosis and Q fever Pub Health Rep *59*, 1363-1373.

to justify the diagnosis of active infection (King et al, 1956). Occasionally, antibodies may be detected in the serum of patients a month after infection, sometimes before the Frei reaction becomes positive The reaction probably remains positive as long as virus is still present in the host. The complement-fixation test is unsatisfactory as an index of the therapeutic effect of drugs. Patients with tertiary lymphogranuloma venereum continue to maintain circulating complement-fixing antibodies even after clinically satisfactory antimicrobial therapy. Diminishing complement-fixing antibody titers cannot be used to establish a retrospective diagnosis even after clinically successful treatment with broad spectrum antimicrobial drugs (Goldberg and Banov, 1956) A Wassermann or Kahn test should always be done; the incidence of biologically false positive serum tests for syphilis in lymphogranuloma is overestimated (Simpson, 1954)

5 Animal experiments used for isolation and identification of the infective agent are experimental rather than practical procedures Suspected material may be inoculated into the yolk sac of an embryonated hen's egg, but intracerebral inoculation of mice is preferred because diagnosis can be made 5 to 15 days earlier Furthermore, the rodents are fairly resistant to infection by bacteria occasionally contaminating specimens of bubo pus or biopsy material Identification of the virus is based on its appearance, the fact that it is more likely to cause disease by the intracerebral than by the intraperitoneal route and its susceptibility to sulfonamides The virus is regularly present in pus from buboes and is more often isolated from tertiary than from primary lesions, even up to 21 years after infection It has been found in the blood, in the spinal fluid in meningeal infection and in the stool of patients with proctitis

Treatment

The results of treatment with sulfonamides have been good, but the serious side-effects have placed an obstacle in the way of their prolonged use. Penicillin, active against experimental lymphogranuloma, proved to be ineffective clinically in the dosages employed. Tetracyclines are effective against experimental infection, and reports on the therapeutic value of chlortetracycline or oxytetracycline in human beings during the early stage of infection were at first enthusiastic, but later ones have been more reserved. Subjective improvement is noted, and patients may be clinically cured in spite of persistence of the virus in the body. For long-standing disease, 500 mg. 4 times a day is recommended The individual dose may be reduced later to 250 mg. and continued for 3 to 6 weeks for early infections and for longer periods for more advanced infections Chlortetracycline is claimed to be less effective than oxytetracycline (Henley, 1953). In spite of long-standing disease, form and function may be remarkably restored. Conversely, treatment may not be wholly effective even in early lymphogranuloma.

Epidemiology

This disease in all probability is of almost world-wide distribution (Favre and Hellerstrom, 1954), but since it is reportable in only a few places there is no way of knowing its prevalence. The deficiency is not imputable to administrative procedure alone, the disease has been poorly reported because satisfactory diagnosis has not been available. The prevalence in tropical countries and in the Mediterranean, southern and eastern ports is less a matter of climate than of unfortunate social conditions Although most of the patients in reported cases have been men, there is no apparent reason why women should not suffer from this infection in about the same

fected in the course of surgical removal of infected lymph nodes, and hospital orderlies have become infected while bathing patients

In the United States there [redacted] no scarcity of cases in clinics and hospitals The disease is reportable in only Alabama, California, Illinois and Washington The greatest number of cases are reported from areas east of the Mississ

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missions to the municipal venereal disease clinic, 1.9 per cent gave strong positive serologic reactions The reservoir of infection among Negroes, shown in 25 to 40 per cent positive Frei tests or complement-fixation reactions in surveys in certain areas of the United States, is a public health problem

to chlortetracycline in psittacosis virus, Virology 4, 156-171
Greaves, A B, and Taggart, S R, 1953, Serology, Frei reaction, and epidemiology of lymphogranuloma venereum, Am J Syph 37, 273-282
Haagen, E, and Mauer, G, 1938, Ueber eine auf den Menschen übertragbare Viruskrankheit bei Sturmvögeln und ihre Beziehung zur Psittakose, Zentralbl Bakt (Abt I) 143, 81-88
Hamre, D, Rake, H, and Rake, G, 1947, Morphological and other characteristics of the agent of feline pneumonitis grown in the allantoic cavity of the chick embryo, J Exper Med 86, 1-6
Hart, J D, and Morgan, H R, 1954, Studies on the factors essential to the initiation and maintenance of multiplication of psittacosis virus (6BC strain) in deficient cells in tissue culture, J Exper Med 99, 461-470
Haussmann, H G, Siegert, R, Ungar, W, and Ruof, H, 1956, Beiträge zur Psittakose- (Ornithose-) Infektion des Menschen Epidemiologische, seuchenhygienische und serodiagnostische Erfahrungen bei einigen Gruppen-Epidemien in Hessen, Arch Hyg 140, 52-78
Heinmets, F, and Golub, O J, 1948, Observations on the growth of psittacosis virus in chorioallantoic membranes by electron microscope, J Bact 56, 509-525
Hellerstrom, [redacted], and Wassen, E, 1930, Meningo-encephalitische Veränderungen bei Affen nach intracerebraler Impfung mit Lymphogranuloma inguinale, Compt rend internat dermat syph, pp 1147-1151
Henles, K S, 1951, Terramycin in early lymphogranuloma venereum, Brit J Ven Dis 29, 36-38
Heyman, A, 1946, The clinical and laboratory differentiation between chancroid and lymphogranuloma venereum, Am J Syph 30, 279-286
Hilleman, M R, 1945, Immunological studies on the psittacosis-lymphogranuloma group of viral agents, J Infect Dis 76, 96-114
Hilleman, M R, Haig, D A, and Helmold, R J, 1951, The indirect complement fixation, hemagglutination and conglutinating complement absorption tests for viruses of the psittacosis-lymphogranuloma venereum group, J Immunol 66, 115-130
Hurst, E W et al, 1953, The therapy of experimental psittacosis and lymphogranuloma venereum (inguinale) II The activity of quinoxaline-1 4-dioxide and substituted and related compounds, with a note on the morphological changes induced in lymphogranuloma virus by these compounds and by antibiotics Brit J Pharmacol 8, 297-305
Irons, J V, Denley M L, and Sullivan, T D, 1955, Psittacosis in turkeys and fowls as a source of human infection in Beaudette, F R (ed), Psittacosis, Diagnosis, Epidemiology and Control, pp 44-65, New Brunswick, N J Rutgers Univ Press
Irons, J V, Mason, D, and White, R F, 1956, Psittacosis (ornithosis) in Texas, South M J 49, 1061-1064
Johnson, R M, and Morgan, H R, 1956, Latent viral infection of cells in tissue culture II Relationship of cell nutrition to initiation of growth of psittacosis virus J Exper Med 103, 703-775
Jørgensen, M B, and Steffensen, K A, 1956, Ornithosis, an analysis of 44 human cases with positive complement fixation tests, Danish M Bull 3, 20-24
Karr, [redacted] V, 1943, Study of latent pneumotropic virus of mice, J Infect Dis 72, 108-116
Karrer, H, Eddie, B, and Schmid, K, 1950, Barnyard fowl as a source of human ornithosis, case report, California Med 73, 55-57
Karrer, H, Meyer, K F, and Eddie, B, 1950, The complement fixation inhibition test and its application to the diagnosis of ornithosis in chickens and in ducks I Principles and technique of the test J Infect Dis 87, 13-23
———, 1950, The complement fixation inhibition test and its application to the diagnosis of ornithosis in chickens and in ducks II Confirmation of the specificity and epidemiological application of the test J Infect Dis 87, 24-36
Katz, E, 1956, The activity of tetracycline on feline pneumonitis virus infection of chick embryos, J Infect Dis 98, 177-186
Kemmerer, G, Haussmann, H G, Schoop, G, and Kauker, E, 1956, Zur Klinik und Epidemiologie der durch Tauben übertragenen menschlichen Ornithose, Deutsche med Wchnschr 81, 930-933
Kempf, A H, Wheeler, A H, and Nungester, W J, 1945, Isolation of an agent belonging to the psittacosis-lymphogranuloma group of viruses, J Infect Dis 76, 135-143
King, A J, Barwell, C F, and Catterall, [redacted] D, 1956, Intradermal tests in the diagnosis of lymphogranuloma venereum, Brit J Ven Dis 32, 207-216
Kissling, R E, Schaeffer, M, Fletcher, O K, Stamm, D D, Bucca, M A, and Sigel, M M, 1956, Diagnosis of psittacosis in parakeets, Pub Health Rep 71, 719-724
Konrád, J, and Strauss, J, 1955, Výskyt ornithosynové formy atypické pneumonie v Československu, Časop lék česk 94, 415-423
Korns, R F, 1955, Psittacosis in ducks and persons exposed to ducks in Beaudette, F R (ed), Psittacosis, Diagnosis, Epidemiology and Control, pp 80-89, New Brunswick, N J, Rutgers Univ Press
Koteen, H, 1945, Lymphogranuloma venereum, Medicine 24, 1-69
Kurotchkin, T J, Libby, R L, Gagnon, E, and Cox, H R, 1947, Size and morphology of the elementary bodies of the psittacosis-lymphogranuloma group of viruses, J Immunol 55, 283-287
Lazarus, A S, and Meyer, K F, 1939, The virus of psittacosis III Serological investigations J Bact 38, 171-198
Levinthal, W, 1930, Die Ätiologie der Psittakosis, Klin Wchnschr 9, 654
Lillie, R D, 1930, Psittacosis rickettsia-like inclusions in man and in experimental animals, Pub Health Rep 45, 773-778
———, 1933, I The pathology of psittacosis in man. [redacted] The pathology of psittacosis in animals and the distribution of Rickettsia psittaci in the tissues of man and animals Nat Inst Health Bull No 161, Washington, D C, pp 1-66

Brand, G, and Lippelt, H, 1954, Zur Herstellung von Antigenen fur die Ornithose (Psittakose)-Komplementbindungsreaktion, Ztschr Hyg *140, 173-179*

Bucca, M A, 1958, Comparison of CF and HI tests on psittacosis-LGV serums Pub Health Rep *73,* 461-464

Buckley, S M, Whitney, E, and Rapp, F., 1955, Identification by fluorescent antibody of developmental forms of psittacosis virus in tissue culture, *Proc Soc Exper Biol & Med 90, 226-230*

Coles, A C, 1930, Micro-organisms in psittacosis, Lancet *1,* 1011-1012

Coles, J D W A, 1940, Psittacosis in domestic pigeons, Onderstepoort J Vet Sc & Animal Ind *15,* 141-148

Connor, W H, Levin, E A, and Ecker, E E, 1937, Observations on the Frei test, J. Infect Dis *60,* 62-63

Coutts, W E, 1950, Lymphogranuloma venereum, a general review, Bull World Health Organ *2, 545-562*

Cox, H R, 1953, Viral and rickettsial toxins, Ann Rev Microbiol *7,* 197-218

———, 1955, Chemotherapy of psittacosis *in* Beaudette, F R (ed), Psittacosis, Diagnosis, Epidemiology and Control, pp. 137-154, New Brunswick, N J, Rutgers Univ Press

Crocker, T T, 1954, The number of elementary bodies per 50% lethal dose of meningo-pneumonitis virus as determined by electron microscopic counting, J Immunol *73,* 1-7

Cuckler, A C, Frost, B M, McClelland, L, and Solotorovsky, M, 1955, The antimicrobial evaluation of oxamycin (D-4-amino-3-isoxazolidone), a new broad-spectrum antibiotic, Antibiotics & *Chemother 5,* 191-197

Dane, D S, 1955, Some observations on the complement-fixation test for the psittacosis-lymphogranuloma venereum group of viruses, M J Australia *1, 382-384*

Davis, D J, 1955, Psittacosis in pigeons *in* Beaudette, F R (ed), Psittacosis, Diagnosis, Epidemiology and Control, pp *66-73,* New Brunswick, N J, Rutgers Univ Press

de Burgh, P, Jackson, A V, and Williams, S E, 1945, Spontaneous infection of laboratory mice with a psittacosis-like organism, Australian J Exper. Biol & M Sc *23,* 107-110

de Gara, P F, and Furth, J, 1948, Pneumonia produced by a meningo-pneumotropic virus, report of a *fatal* case, with observations on the interrelationship of psittacosis-like viruses, Arch Path *45,* 474-493.

Eaton, M D, Beck, M D, and Pearson, H E, 1941, A virus from cases of atypical pneumonia,

50, 291-295

Enright, J B, and Sadler, W. W, 1954, Presence in human sera of complement-fixing antibodies to virus of sporadic bovine encephalomyelitis, Proc. Soc. Exper. Biol & Med. *85,* 466-468

Ephrati-Elizur, E, and Bernkopf, H., 1956, Isolation of six strains of ornithosis virus from children with infections of the respiratory tract, J Infect Dis *98,* 45-51

Favre, M, and *Hellerstrom, S, 1954, The epidemiology,* aetiology and prophylaxis of lymphogranuloma inguinale, Acta dermato-venereol (Suppl 30) *34,* 1-68

Findlay, G M, *1938,* I Inclusion bodies and their relationship to viruses *in* Doerr, R, and Hallauer, C., Handbuch der Virusforschung, pp *292-368,* Vienna, Springer, I Hälfte.

Fitz, R H, Meiklejohn, G, and Baum, M D, 1955, Psittacosis in Colorado, Am J M Sc. *229, 252-*261

Francis, T, Jr, and Magill, T P, 1938, An unidentified virus producing acute meningitis and pneumonitis in experimental animals, J Exper. Med *68,* 147-160

*Frei, W, 1925, Eine neue Hautreaktion bei "Lym*phogranuloma inguinale," Klin Wchnschr *4,* 2148-2149

Gaylord, W H, Jr, 1954, Intracellular forms of meningopneumonitis virus, *J* Exper Med *100,* 575-580

Gerloff, R K, and Lackman, D B, 1954, Observations regarding the presence of psittacosis and related viruses in the northwestern states, Am *J* Pub Health *44, 323-327*

Giroud, P, 1956, Ce que la clinique et l'etude sérologique systématique nous ont appris sur les maladies de l'homme provoqueés par les éléments néo-rickettsiens situes à côte du groupe de la psittacose, Laval med *21,* 535-554

Giroud, P, and *Jadin, J, 1954,* Premiers resultats concernant le virus des Bashi isole dans la province du Kivu au Congo belge, Bull Soc path exot *47,* 578-588

Giroud, P, Roger, F, and Dumas, N, 1955, Contribution à l'étude des néo-rickettsioses L'évolution des anticorps au cours des diverses maladies de l'homme et des animaux Bull Soc path exot *48,* 21-24

Gogolak, F M, 1953, The histopathology of murine pneumonitis infection and the growth of the virus in the mouse lung, J Infect Dis *92, 254-272*

———, 1954, The mouse erythrocyte hemagglutinin of feline pneumonitis virus, J Infect Dis *95,* 220-225

Gogolak, F M, and Ross, M R, 1955, The properties and chemical nature of the psittacosis virus hemagglutinin, Virology *1,* 474-496

Goldberg, J, and Banov, L., Jr, 1956, Complement-*fixation titres in tertiary lymphogranuloma* venereum; a study of results after treatment with broad-spectrum antibiotics, Brit J Ven Dis *32,* 37-39

Gonnert, R, 1941, *Die Bronchopneumonie, eine neue* Viruskrankheit der Maus, Zentralbl Bakt *(Abt* I) *147,* 161-174

Gordon, F B, Andrew, V. W, and Wagner, J C, 1957, Development of resistance to *penicillin and*

Omori, T et al, 1953, *Study of an infectious pneumonia of goat caused by a virus I Isolation of the causative agent and its characteristics* Japan, Government Experimental Station for Animal Hygiene, Tokyo, Reports, No *27*, pp 101-119

Palmer, W L, Kirsner, J B, and Rodaniche, E C, 1943, Studies on lymphogranuloma venereum infection of the rectum, J A M A *118*, 517-524

Pate, D D, Boney, W A, Jr., and Delaplane, J P., 1954, Turkey ornithosis I A report of natural and experimental infections J Am Vet M A *125*, 476-478

Philip, C B, 1955, There's always something new under the "parasitological" sun (the unique story of the helminth-borne salmon poisoning disease), J Parasitol *41*, 125-148

Philip, C B, Hadlow, W J, and Hughes, L E, 1953, *Neorickettsia helminthoeca*, a new rickettsia-like disease agent of dogs in western United States transmitted by a helminth International Congress for Microbiology, 6th, Rome, 1953, Summaries of communications, vol *2*, pp 256-257

Pollard, M, 1955, Psittacosis in seashore birds in Beaudette, F R (ed), Psittacosis, Diagnosis, Epidemiology and Control, pp 99-104, New Brunswick, N J, Rutgers Univ Press

Pollard, M, Caplovitz, C D, and Swausch, C D, 1947, The identification of an ornithosis virus from the willet, Texas Rep Biol & Med *5*, 337-340

Prouty, R L, and Jordan, W S, Jr, 1956, A family epidemic of psittacosis with occurrence of a fatal case, A M A Arch Int Med *98*, 365-371

Rake, G, 1947, The initial body and the plaque form in the *Chlamydozoaceae*, J Bact *54*, 635-640

Rake, G, and Jones, H P, 1942, Studies on lymphogranuloma venereum I Development of the agent in the yolk sac of the chicken embryo J Exper Med *75*, 323-338

———, 1944, Studies on lymphogranuloma venereum II The association of specific toxins with agents of the lymphogranuloma-psittacosis group J Exper Med *79*, 463-486

Rake, G, McKee, C M, and Shaffer, M F, 1940, Agent of lymphogranuloma venereum in the yolk-sac of the developing chick embryo, Proc Soc Exper Biol & Med *43*, 332-334

Rivers, T M, and Schwentker, F F, 1934, Vaccination of monkeys and laboratory workers against psittacosis, J Exper Med *60*, 211-238

Robinson, H M, 1940, Analysis of intracutaneous tests on 309 patients for lymphogranuloma venereum and chancroid, South M J *33*, 144-154

Roca-Garcia, M, 1949, Viruses of the lymphogranuloma-psittacosis group isolated from opossums in Colombia, opossum virus A, J Infect Dis *85*, 275-289

Ross, M R, and Gogolak, F M, 1957, The antigenic structure of psittacosis and feline pneumonitis viruses I Isolation of complement-fixing antigens with group and species specificity Virology *3*, 343-364

———, 1957, The antigenic structure of psittacosis and feline pneumonitis viruses II Chemical nature of the alkali-soluble antigens Virology *3*, 365-373

Rubin, H, 1954, A disease in captive egrets caused by a virus of the psittacosis-lymphogranuloma venereum group, J Infect Dis *94*, 1-8

Seibert, R H, Jordan, W S, Jr., and Dingle, J H., 1956, Clinical variations in the diagnosis of psittacosis, New England J Med *254*, 925-930

Simpson, R G, 1954, The relation of lymphogranuloma venereum to syphilis and to falsely positive serologic tests for syphilis, Am J Syph *38*, 422-428

Smadel, J E, Wertman, K, and Reagan, R L, 1943, Yolk sac complement fixation antigen for use in psittacosis-lymphogranuloma venereum group of diseases, Proc Soc Exper Biol & Med *54*, 70-74

Stamp, J T, 1951, Developmental forms of the virus of ovine enzootic abortion, J Comp Path *61*, 215-218

Stamp, J T, McEwen, A D, Watt, J A A, and Nisbet, D I, 1950, Enzootic abortion in ewes I Transmission of the disease Vet Record *62*, 251-254

Strauss, J, 1956, Virologický průkaz ornithosy u lidí a kachen v Československu Česk Epidemiol, Mikrobiol, Imunol *5*, 281-291

Strobel, W, 1954, Beitrag zum Krankheitsbild der Ornithose im Kindesalter, Deutsche med Wchnschr *79*, 176-178

Swain, R H, 1955, A microscopical study of the reproduction of psittacosis virus, Brit J Exper Path *36*, 507-514

Van Rooyen, C E, 1955, The early history of psittacosis in Beaudette, F R (ed), Psittacosis, Diagnosis, Epidemiology and Control, pp 1-8, New Brunswick, N J, Rutgers Univ Press

Volkert, M, and Christensen, P M, 1955, Two ornithosis complement-fixing antigens from infected yolk sacs. I The phosphatide antigen, the virus antigen and methods for their preparation Acta path et microbiol scandinav *37*, 211-218

Volkert, M, and Matthiesen, M, 1956, An ornithosis-related antigen from a coccoid bacterium, Acta path et microbiol scandinav *39*, 117-126

Wagner, J, and Victor, J, 1953, Psittacotic lesions and respiratory immunity in intradermally vaccinated guinea-pigs, Am J Path *29*, 155-173

Wagner, J C, Golub, O J, and Andrew, V W, 1949, Cross immunity studies between virus strains in the psittacosis-lymphogranuloma venereum group, J Infect Dis *84*, 41-46

Wagner, J C, Meiklejohn, G, Kingsland, L C, and Hickish, H W, 1946, Psittacosis vaccines prepared from chick embryo tissues J Immunol *54*, 35-46

Walton, K W, 1954, The pathology of a fatal case of psittacosis showing intracytoplasmic inclusions in the meninges, J Path & Bact *68*, 565-572

Ward, C G, and Birge, J P, 1952, Psittacosis (ornithosis) following contact with pheasants, report of a case, J A M A *150*, 217-219

Ward, C G, Hiddinger, A L, Morrissey, R A, and Birge, J P, 1954, Psittacosis-lymphogranuloma venereum virus antibodies in man, experiences with thirty-seven persons with respiratory disease

Lippelt, H, and Brand, G, 1955, Die Komplementbindungsreaktion in der Diagnostik der Ornithose (Psittakose), Deutsche med. Wchnschr *80*, 110-114

Loosli, C G, and Ritter, M H, 1948, The pathogenesis and histopathology of air-borne pneumonitis virus infection in mice the effect of penicillin G upon developing lesions, J Clin Invest *27*, 547

Luger, N M, 1950, The incidence of lymphogranuloma venereum as determined by the quantitative complement-fixation test, Am J Syph *34*, 351-355

Lyon, [redacted], 1956, Virus Diseases and the Cardiovascular System, a Survey, [redacted] 83-89, New York, Grune (215 pp)

McEwen, A D, and Foggie, A, 1956, Enzootic abortion in ewes, prolonged immunity following the injection of adjuvant vaccine, Vet Record *68*, 686-689

McKercher, D. G, 1952, A virus possibly related to the psittacosis-lymphogranuloma-pneumonitis group causing [redacted] pneumonia in sheep, Science *115*, 543-545

Mandel, A, and Jordan, W S, Jr, 1952, Ornithosis (psittacosis) in chickens and poultry workers, Am J Hyg *55*, 230-238

*Manire, G P, and Meyer, K F, 1950, The tox*ins of the psittacosis lymphogranuloma group of agents II Effect of aureomycin and penicillin on the toxins of psittacosis viruses J Infect Dis *86*, 233-240

Mason, D M, 1957, Unpublished data

Matumoto, M et al, 1955, Studies on the disease of cattle caused by a psittacosis-lymphogranuloma group virus (Miyagawanella) VI Bovine pneumonia caused by this virus Jap J Exper Med *25*, 23-34

Menges, R W, Harshfield, G S, and Wenner, H A, 1953, Sporadic bovine encephalomyelitis, studies on pathogenesis and etiology of the disease, J Am Vet M A *122*, 294-299

Meyer, K F, 1940, The host-parasite relationship in the heterogenous infection chains, Harvey Lectures, *ser 35 (1939-40), pp 91-134*

———, 1941a, Phagocytosis and immunity in psittacosis, Schweiz med Wchnschr *71*, 436-438

———, 1941b, Pigeons and barn yard fowls as possible sources of human psittacosis or ornithosis, Schweiz med Wchnschr *71*, 1377-1379

———, *1942, The ecology of psittacosis* and ornithosis, Medicine *21*, 175-206

———, 1952, Ornithosis and psittacosis *in* Biester, H E, and Schwarte, L H (eds), Diseases of Poultry, ed 3, pp 569-618, Ames, Iowa, Iowa State College Press.

———, 1953, Psittacosis group, Ann New York Acad Sc *56*, 545-556

———, 1954, Early diagnosis of infections by the psittacosis-lymphogranuloma venereum group *in* Hartman, F W, Horsfall, F. L, Jr, and Kidd, J G (eds), The Dynamics of Virus and Rickettsial Infections, pp 295-323, New York, Blakiston

———, 1955, Problems in the control of psittacosis and ornithosis, Am Vet M A, Proc 92 Annual Meet, pp 412-419

Meyer, K. F, and Eddie, B, 1942, Spontaneous ornithosis (psittacosis) in chickens the cause of a human infection, Proc Soc Exper. Biol & Med. *49, 522-525*

———, *1951*, Human carrier of the psittacosis virus, J Infect Dis *88*, 109-125

———, *1952*, Reservoirs of the psittacosis agent, Acta Tropica *9*, 204-215

———, 1953, Characteristics of a psittacosis viral agent isolated from a turkey, Proc Soc Exper Biol & Med *83*, 99-101

———, 1956a, Psittacosis *in* Diagnostic Procedures for Virus and Rickettsial Diseases, ed *2*, pp 399-430, New York, Am Pub. Health Assoc

———, 1956b, Unpublished data

Meyer, K F, *Eddie, B, Richardson, J H, Shipko*witz, N L, and Muir, R, 1958, Chemotherapy [redacted] Beaudette, F R (ed), Progress in Psittacosis Research and Control, pp 163-196, New Brunswick, [redacted] J, Rutgers Univ Press

Meyer, K F, Eddie, B, and Yanamura, H, 1942, Active immunization to Microbacterium multiforme psittacosis in parrakeets and ricebirds, J Immunol *44, 211-217*

Michail, C P, 1936, Studies on ornithosis in Greece, Acta microbiol hellen *1*, 76-81

Miyagawa, Y, Mitamura, T, Yaoi, H, Ishii, N, and Okanishi, J, 1935, Studies on virus of lymphogranuloma inguinale *Nicolas, Favre* and *Durand* II, III, IV, V Jap J Exper Med *13*, 331-339, *723-750*

Mohr, W., 1954, Untersuchungen und Beobachtungen zur Verbreitung und Klinik der Ornithose *(Psittakose)* in Deutschland, Ztschr ges inn Med *9*, 1005-1010.

Monsur, K. A, and Barwell, C F, 1951, Observations on the antigenic relationship between the virus of enzootic abortion in ewes and viruses of the psittacosis-lymphogranuloma group, Brit J Exper Path *32*, 414-421

Morgan, [redacted] R, 1954, Factors related to the growth of psittacosis virus (strain 6BC) IV Certain amino acids, vitamins, and other substances J Exper Med *99*, 451-460

—

103, 37-47

Moulder, J W, 1954, Biochemical aspects of the growth *of feline pneumonitis virus* in the chick embryo yolk sac, Bact Rev *18*, 170-176

Moulder, J W, and Weiss, E, 1951, Purification and properties of the agent of feline pneumonitis, J Infect Dis *88*, 56-67

Mykytowycz, R, Dane, D S, and Beech, M, 1955, Ornithosis in the petrel, *Puffinus tenuirostris* (Temminck), Australian J Exper Biol & Med Sc *33*, 629-636

Nigg, C, and Eaton, M D, 1944, Isolation from

Ol

Rep. *59*, 1299-1311

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34

Trachoma and Inclusion Conjunctivitis

INTRODUCTION

Trachoma and inclusion conjunctivitis are closely related but distinct viral infections of the external eye, often similar in clinical appearance in their early stages but differing widely in clinical course and economic importance Trachoma has a world-wide distribution, rarely heals spontaneously, invariably *involves the cornea and usually* leads to visual disability from conjunctival and corneal cicatrization Inclusion conjunctivitis never involves the cornea or produces scars, is always self-limited and never has been found in certain populations Moreover, trachoma affects the eye only, whereas inclusion conjunctivitis, *seen most often as one type of ophthalmia* neonatorum, is the ocular manifestation of a benign nongonococcal urethritis and cervicitis

In spite of these important differences, the two diseases have often been confused because of the morphologic identity of their inclusion bodies and because follicular hypertrophy of the conjunctiva is a prominent clinical sign in both These similarities, both clinical and etiologic, have influenced investigators to study the two infections simultaneously, hence their consideration in the same chapter in the present volume

TRACHOMA

(SYNONYM Granular conjunctivitis)

HISTORY

Trachoma was an affliction of antiquity and one of the first disease entities to be described [illegible]

cedures (grattage, scarification of follicles, and application of copper salts) that have been in use until recent times Trachoma was common in ancient Rome and is known to have afflicted Cicero, Horace and Pliny the [illegible]

out Europe

Of all ocular affections, trachoma has always been the one of greatest world-wide economic and social consequence It is the only eye disease in whose interest special hospitals and research institutes have been established the world over; to which a special journal, the *Revue Internationale du Trachome*, is devoted, and toward whose eradication the World Health Organization has instituted an Expert Panel and sponsored a series of mass treatment campaigns. Several of these are presently under way in a number of countries, but in spite of their partial success the disease is still a major affliction throughout the world

The highest incidence of trachoma prevails in North Africa, Egypt and the Middle East where the majority of the population is still affected It is also widespread in all the other Mediterranean countries, as well as in the Balkans, in Russia and throughout the Orient In North European countries it has almost

having significantly high antibody titers, J A M A *155,* 1146-1150

Weiss, E, 1949, The extracellular development of agents of the psittacosis-lymphogranuloma group (*Chlamydozoaceae*), J Infect Dis *84,* 125-149

———, 1955, The nature of the psittacosis-lymphogranuloma group of microorganisms, Ann Rev Microbiol *9,* 227-252

Weyer, F, and Lippelt, H, 1956, Ein Beitrag zur Frage der Tauben-Ornithose in Deutschland, Ztschr Hyg *143,* 223-246

Whitney, E, and Gnesh, G M, 1954, Potent psittacosis antigens free of anticomplementary activity, Proc Soc Exper Biol & Med *87,* 356-360

Yanamura, H Y, and Meyer, K F, 1942, Active immunity to Microbacterium multiforme psittacosis in the mouse, J Immunol *44,* 195-209

Yeatman, C, and McEwin, J, 1945, Infections with psittacosis in Adelaide, M J Australia *2,* 109-114

York, C J, and Baker, J A, 1956, *Miyagawanella bovis* infection in calves, Ann New York Acad Sc *66,* 210-214

Yow, E M, Preston, J, and Leachman, [redacted] D, 1957, Hepatitis due to the psittacosis virus occurring during an epidemic among turkey handlers, Am J Med *22,* 978-979

Zahler, [redacted] A, and Moulder, J W, 1953, The incorporation of radioactive phosphate into feline pneumonitis virus in the chick embryo yolk sac, J Infect Dis *93,* 159-165.

Zichis, J, and Shaughnessy, H J, 1945, Isolation of an apparently new virus from two fatal pneumonia cases, Science *102,* 301-302.

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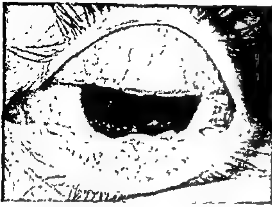


FIG 116 Trachoma, Stage I, in an Indian child, showing follicular hypertrophy of the conjunctiva.

disappeared. In the United States it is still prevalent among the Indians and has in fact recently been recrudescent, particularly among the tribes of the Southwest In the white population of the United States it used to flourish among the mountain people along the so-called "Daniel Boone Trail" across the Appalachians, but the incidence in this group has steadily decreased. Sporadic cases are seen elsewhere in the country, especially among immigrants in the form of reactivations of old cicatricial disease.

In 1907 Halberstaedter and Prowazek found the elementary bodies and the cytoplasmic inclusions that characterize the disease, and in 1910 Lindner described the initial-body form of the virus. In 1912 Nicolle et al. reported the first positive filtration experiments which they accomplished with modified Berkefeld V filters on *M. sylvanus* monkeys. In 1935 Thygeson et al. performed a human inoculation experiment with trachomatous material filtered through a gradocol membrane of 0.6 micron average pore diameter and found that infectivity was associated with the presence of elementary bodies in the filtrate Subsequent investigators noted the many resemblances of trachoma virus to the viruses of psittacosis and lymphogranuloma venereum On this basis it was classified by Bedson and Rake, along with inclusion conjunctivitis virus, as a member of the psittacosis-lymphogranuloma group

Trachoma virus studies have been greatly handicapped by the fact that so far it has been impossible to cultivate the virus in series or in quantity in tissue culture or in the chick embryo,* and by the fact that the only susceptible animals (monkeys, baboons and apes) develop a self-limited chronic follicular conjunctivitis which lacks the diagnostic pannus and cicatrization of the human disease. The recognition in 1938 by Loe and others that trachoma was susceptible to the sulfonamides marked the first major advance in therapy since the time of Hippocrates

CLINICAL PICTURE

The incubation period of trachoma, as determined from experimental human inoculations, is from 5 to 7 days. The onset is usually insidious, particularly in children, but it may be acute, depending apparently on the amount of inoculum. The onset in adult infections and experimental infections has almost always been acute and has sometimes been fulminating, with chemosis of the bulbar conjunctiva and copious exudate. The more common childhood disease may be extremely mild in its early stages Indeed, it may present no gross signs whatever beyond perhaps a slight ptosis; however, when the lids are everted, a characteristic follicular hypertrophy (Fig 116), most marked on the upper tarsal conjunctiva, is to be seen In these early cases biomicroscopic examination is required to detect the corneal signs of the disease which include epithelial keratitis, subepithelial infiltration and extension of limbal vessels (pannus) In cases with acute or subacute onset, the follicles are often masked by intense papillary hypertrophy of the conjunctiva, but the corneal changes (edema of the upper limbus, subepithelial infiltration, pannus) appear early and are conspicuous This acute stage, with its abundant exudate, lasts for several weeks and is followed by a chronic stage of variable intensity; the exudate dwindles to a minimum (unless there is secondary infection), and the symptoms of irritation subside

Whether the onset has been insidious or acute, the disease progresses in much the same way over a period of months or years to conjunctival and corneal cicatrization, except that trachoma contracted early in life, as is the rule in such heavily infected areas as Egypt and the Middle East, tends to be a milder disease and to heal spontaneously more often. The degree of cicatricial change varies greatly, but lid deformities, including ptosis, trichiasis and entropion, are common, and in old cases visual disturbance from corneal scars is almost the rule In unusually severe cases

* See footnote page 733

cicatrization may destroy tear function, then keratitis sicca, with keratinization of the corneal epithelium, may ensue. Secondary bacterial infection, so common in the Middle East, may further increase corneal ulceration, and vision may be totally lost Since no immunity develops in trachoma, reinfection of healed cases occurs and is a major problem in heavily infected countries.

Pathologic Picture

The earliest recognizable pathologic sign of trachoma is the characteristic cytoplasmic inclusion body in conjunctival and corneal epithelial cells (Wilson, 1937) This is followed by subepithelial infiltration with small

Advanced stages are characterized by cell necrosis and finally by cicatrization All the pathologic changes in trachoma affect the upper half of the conjunctival sac and the cornea more prominently than the lower half

Although mononuclear cells dominate the subepithelial infiltration, the exudate is composed chiefly of polymorphonuclear cells This is particularly true in the acute stages of the disease and in no way depends on secondary bacterial infection In contradistinction, the exudates of conjunctival infections with typical viruses, such as the adenoviruses and the herpes simplex virus, are characteristically mononuclear.

The inclusion bodies vary in number according to the clinical severity of the disease and are most numerous in scrapings from the upper tarsal and the upper limbal regions, i e, from the areas of maximum disease intensity They are most abundant in the superficial layers of the epithelium, are rare in the basal layers and never occur in follicle cells or in the subepithelial layers

Experimental Infection, Host Range

The only experimental animals susceptible to trachoma virus are, in the order of their susceptibility, apes, baboons and monkeys Unfortunately, none of these animals develops pathologic changes characteristic of human trachoma, i e, pannus and cicatrization. Moreover, the experimental disease they do develop is self-limited and appears only as a follicular conjunctivitis which cannot be differentiated with certainty from the follicular conjunctivitis produced by inclusion conjunctivitis virus, or even from the spontaneous folliculosis with which monkeys, baboons and apes are frequently affected (Wilson, 1930, Bland, 1945) The inclusion bodies characteristic of trachoma have been found regularly in the experimental disease in apes, exceptionally in the disease in baboons and never in the disease in monkeys This paucity of inclusions is believed to be due to the mildness of the experimental disease in which there are minimal inflammatory signs and exudate However, that the disease is actually trachoma has been established by Nicolle et al. 1912 and by Bland (1944) These workers succeeded in transferring the experimental disease from its animal hosts to human volunteers who in turn developed typical trachoma

Experimental infection in human beings has been achieved on numerous occasions, usually with an acute onset and abundant inclusions No immunity to reinfection has developed in either human or animal hosts

Etiology

The cause of trachoma is a virus of large particle size resembling the viruses of psittacosis and lymphogranuloma venereum in staining properties, particle size, intracellular cycle of morphologic variation, and susceptibility to sulfonamides and antibiotics. Its elementary bodies measure about 0 25 micron in diameter in Giemsa-stained preparations They are to be seen in conjunctival and corneal epithelial cells in colony form (the inclu-

cells exclusively It undergoes an intracellular cycle of morphologic variation in which the young forms (the initial bodies) are larger, typically oval, stain bipolarly a pure blue with Giemsa, and occur in colony form as initial body inclusions (Fig 117B) In wet-fixed slides or free in the exudate the initial bodies vary in size from 0.3 to 1 2 microns and exhibit typical division forms Mitsui and Suzuki (1956) have studied them under the electronmicroscope (Fig 118) and noted their lesser density as compared with the density of the elementary bodies. While not denying their division by binary fission, these

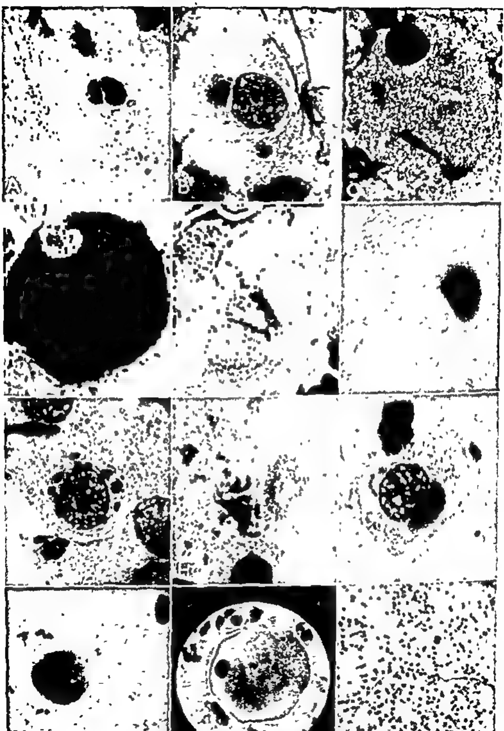


FIG. 117 (A) Free elementary bodies in the exudate from a case of severe trachoma. Giemsa stain $\times 1500$.
(B) Young inclusion bodies made up of large swollen forms, the so-called initial bodies of Lindner. Giemsa stain. $\times 1500$. (*Continued on facing page*)

observers have suggested the possibility of an alternate vegetative process of reproduction

The inclusion body is a virus colony in which the elementary and initial bodies are embedded in a carbohydrate matrix composed principally of glycogen (Figs. 117C-F) (Rice, 1936). The evolution of the inclusion body in a susceptible cell requires about 48 hours Multiple infection of cells is common (Fig 117G) The existence of a toxin is suggested by the following facts (1) toxins have been demonstrated for other members of the psittacosis-lymphogranuloma venereum group of agents (Rake and Jones, 1944), (2) in the experiment of Mitsui, et al (1954), repeated instillations of a virus-free ultrafiltrate of trachomatous material produced follicular hypertrophy of the conjunctiva of a volunteer; and (3) important subepithelial changes occur in trachoma even though the virus appears to be limited strictly to the epithelial layer In this connection it is noteworthy that attempts to infect the subepithelial layers of the conjunctiva by injecting trachoma virus through the skin of the lids failed, and that subsequent direct inoculation of the conjunctiva of the same subjects succeeded (Michail and Vancea, 1932).

The virus is rapidly inactivated by bile and by drying, by alternate freezing and thawing and by incubator temperature (37° C.). Its thermal death point is 45° C maintained for 15 minutes (Julianelle, 1938) At refrigerator temperature, or in 50 per cent glycerol, it can be preserved for as long as a week. Sulfanilamide has no virucidal effect in vitro (Julianelle and Smith, 1942). Attempts to cultivate the virus in tissue culture in quantity or in series have been unsuccessful, even when cultures of human conjunctival and corneal epithelium have been used Trachomatous conjunctival epithelium has rapidly lost its infectivity for baboons when grown in tissue culture (Thygeson, 1939) There are numerous claims for cultivation of the virus in the yolk sac and the chorioallantois of the developing chick embryo (Macchiavello, 1944, Poleff, 1949, Stewart and Badir, 1950), but cultivation in series or in quantity on these membranes has certainly not yet been achieved.* Low titers of group

* Since the preparation of this chapter, cultivation of trachoma virus in series on the yolk sac of the developing chick embryo has been claimed by Tang, F F, Chang, H L, Huang, Y T, and Wang, K C Studies on the etiology of trachoma with special reference to isolation of the virus in chick embryo (Chinese M J, 75, 429-447, 1957) and confirmed by Collier, L H, and Sowa, J, Isolation of trachoma virus in embryonate eggs (Lancet, 1, 993-996, 1958) Collier and Sowa produced trachomatous infection of a human volunteer with the yolk-sac-cultivated material and recovered characteristic inclusions and elementary bodies from the experimental disease

Fig 117 (*Continued from facing page*)

(C) Mature inclusion body in which the cytoplasm of the epithelial cell has been entirely replaced by elementary bodies Giemsa stain ×1500

(D) Epithelial cell (shown in C) previously stained by Lugol's solution to show the carbohydrate matrix which takes a reddish-brown coloration with iodine

(E) An inclusion bo[illegible] showing both elementary and initial bodies Giemsa stain ×1500

(F) Epithelial cell [illegible] to show the carbohydrate matrix [illegible] he honeycomblike arrangement of the matrix in which th[illegible] es, not af[illegible] the iodine, are embedded ×1500

(G) [illegible] epithelial cell Giemsa stain ×1500

(H [illegible] in a newborn infant

(I) Inclusio[illegible] conjunctivitis Giemsa stain ×1500

(J) Free elementary and initial bodies in the exudate from a severe case of inclusion conjunctivitis Giemsa stain ×1400

(K) Inclusion body in cervical epithelium from the mother of a baby with inclusion conjunctivitis Giemsa stain ×1200

(L) Free elementary bodies in cervical secretion from a woman whose child developed inclusion conjunctivitis Giemsa stain ×1750

complement-fixing antibodies for the psittacosis-lymphogranuloma venereum group have been demonstrated in sera from trachoma patients by Rake et al (1942), but there is no evidence to suggest that they are concerned in the clinical course of the disease or in developing immunity to it. Monkeys and baboons that have recovered from experimental trachoma show no immunity to reinfection, and the second infection runs the same clinical course as the first.

The virus filters with difficulty and only through such coarse filters as the Berkefeld V candle and gradocol membranes of 0 6 micron A P.D. or above. If an active filtrate is to be obtained, the elementary bodies must pass the filter. This was demonstrated in the experiment of Thygeson et al. (1935) in which a human volunteer was inoculated successfully with a gradocol filtrate (0 6 micron A.P.D.) The original material, consisting of ground epithelial scrapings from trachomatous Indian children, contained abundant inclusions Elementary bodies were demonstrated in the bacteria-free filtrate after centrifugation, and inclusion bodies were numerous in the experimental disease at onset.

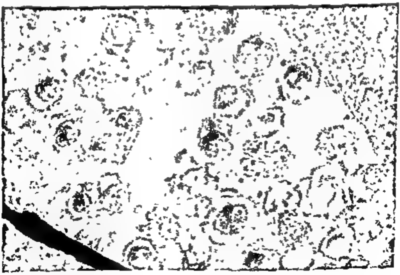
Diagnosis

Trachoma can usually be diagnosed on the basis of the following clinical signs (1) follicle formation, most prominent on the upper tarsal region, (2) trachomatous pannus, which can be recognized in its incipience early in the disease on slit-lamp examination of the upper limbal region, and (3) conjunctival cicatrization

Laboratory diagnosis is based on the demonstration of cytoplasmic inclusion bodies and on certain cytologic changes in expressed follicular material Although the inclusions are morphologically identical with those of inclusion conjunctivitis, in trachoma they are more numerous on the upper tarsal conjunctiva than on the lower, and in inclusion conjunctivitis they are more numerous on the lower tarsal conjunctiva than on the upper (Braley, 1940). In expressed follicular material from trachoma, necrotic changes not found in other follicular diseases of the conjunctiva are to be seen in the form of cell debris, pale-staining cells, and numerous macrophages loaded with cell fragments (Thygeson, 1946).

Treatment

Prior to the introduction of sulfonamide therapy in 1938, trachoma was treated by a combination of medical and surgical means Medical treatment consisted in the control of secondary infection by antiseptic drops, and in the application to the conjunctiva of caustics, such as copper sulfate, silver nitrate,



... section show-

quinine bisulphate, etc. Surgical treatment consisted in the correction of cicatricial deformities, such as entropion and trichiasis, and in the removal of diseased conjunctiva by tarsectomy The inadequacy of these measures is indicated by the fact that few patients tolerated treatment sufficiently long to obtain relief and that the few cures obtained took from one to many years of continuous medical care

Under sulfonamide therapy the prognosis of trachoma has changed entirely In early cases cures can now be expected in a matter of weeks No sulfonamide resistance has ever been observed As in the related disease, lymphogranuloma venereum, low dosages and relatively prolonged treatment times (2 to 4 weeks) are required. In the cicatricial stages sulfonamide therapy is less satisfactory, and surgical intervention for the relief of cicatricial complications may be required Prognosis is poorest in those cases in which tear-function has been lost and in cases complicated by vernal catarrh or secondary bacterial infection

The broad-spectrum antibiotics (chlortetracycline, chloramphenicol and oxytetracycline) have all shown antitrachomatous activity (Mitsui et al, 1951, Bietti, 1951) When used orally, their effect has been comparable with that of the sulfonamides, but when used topically they appear to be more efficacious than topically administered sulfonamides

Epidemiology

In the countries of the Middle East, such as Egypt, trachoma is commonly acquired in the first year of life, usually from the mother When periodic epidemics of the acute ophthalmias occur, it is often transmitted simultaneously with the bacterial infections (Wilson, 1930) In countries in which the disease is only endemic, as in the United States, spread occurs only when there is constant exposure to infection under conditions of poor hygiene such as formerly obtained among the mountain folk of West Virginia, Kentucky and Tennessee and still prevail in some of our Indian populations

On the basis of the virus content of epithelial scrapings, it may be assumed that acute cases are highly infectious and chronic cases only slightly so In sporadic cases it is often impossible to trace the source of infection, but it is believed that eye-to-eye transmission by means of fingers or fomites is possible and that the fly may play an important role (Wilson, 1930) So far no evidence to indicate the existence of a subclinical infection or carrier state has been advanced

Control Measures

The control of trachoma in the countries in which it is pandemic devolves chiefly upon the control of the acute ophthalmias (Wilson, 1945) This is now theoretically possible by means of chemotherapy but on practical grounds is limited by lack of funds, the countries in which trachoma is widespread being the ones most economically depressed Since trachoma is a disease of filth and poor personal hygiene, all measures leading to improvement in the economic condition of the population exert a prophylactic effect In the United States the experience of the Division of Indian Health in its attempts to control the disease has illuminated the dramatic effect of the change in therapeutic method Prior to the introduction of the sulfonamides in 1938, the extensive antitrachoma campaign conducted in the Indian schools was only moderately successful, but when sulfonamide therapy was instituted, the incidence of the disease was strikingly reduced in short order (Forster and McGibony, 1944) However, a recent recrudescence, particularly among the Indians of the Southwest, has necessitated a revival of the diagnostic and treatment campaigns In the white population of the United States control consists in the early recognition and treatment of the disease, particularly in isolated communities of the old "trachoma belt" To this end stationary and mobile trachoma clinics have been established in Arkansas, Illinois, Missouri and Virginia

INCLUSION CONJUNCTIVITIS

(Synonyms Inclusion blennorrhea, paratrachoma, swimming-pool conjunctivitis)

History

A benign form of conjunctivitis in the newborn, unassociated with pathogenic bacteria, was described by Morax as early as 1903 Shortly after Halberstaedter and Prowazek discovered cytoplasmic inclusion bodies in trachoma in 1907, Stargardt, 1909, and then

Schmeichler, 1909, noted identical inclusions in ophthalmia neonatorum. In 1911 Lindner reported finding inclusions in all but a very few cases of ophthalmia neonatorum of nonbacterial origin He had been able to transmit the disease to the conjunctiva of the baboon and to demonstrate identical inclusion bodies in the experimental disease. He defined its clinical characteristics and named it *Einschlussblennorrhoe* (inclusion blennorrhea). In 1910 Wolfrum described two successful inoculations of human beings with inclusion blennorrhea exudate.

Searching for the origin of this disease of the newborn, Halberstaedter and Prowazek, 1909, found typical inclusions in scrapings from the genito-urinary tracts of mothers of diseased infants and postulated the existence of a genito-urinary inclusion disease of the male and the female This theory was supported by Lindner, 1910b and Heymann, 1910, who found inclusions in several cases of urethritis in the male In the same year Fritsch et al, 1910, produced a conjunctival infection in a baboon with urethral exudate from nongonorrheal urethritis containing inclusions.

The first filtration experiments were made in 1912 Botteri, 1912, induced infection in the eye of a baboon with a Berkefeld V filtrate, and Gebb, 1914, confirmed his work by obtaining infection in a human subject with a similar filtrate Subsequent filtration studies by Thygeson (1934), Tilden and Gifford (1936), and Julianelle et al (1938) showed that the virus was filterable with relative ease through coarse Berkefeld candles and gradocol membranes

Unfortunately, some confusion arose as to the relationship of the disease to trachoma and to the conjunctivitis with inclusions seen in adults, particularly in bathers in certain swimming-pools Some authors regarded this adult disease as identical with trachoma, but such a conclusion was obviated by the self-limited nature of the inclusion disease and its failure to produce pannus or cicatrization. Morax (1933) differentiated it sharply from trachoma and offered evidence, later confirmed by Thygeson (1934) and Julianelle (1937), to show that the infant and the adult types of conjunctivitis with inclusions, in spite of certain differences, were manifestations of a single disease

Thygeson and Mengert (1936) found that in mothers of babies with inclusion blennorrhea, the genito-urinary disease was limited to the external os of the cervix and to an area of transitional epithelium identical histologically with the epithelium of the conjunctiva. They reported that the infection produced no clinical symptoms in the mothers, but that in the fathers it was only occasionally subclinical and usually appeared as a mild, nonspecific urethritis of several months' dura-

to be an occupational disease of obstetricians

sponded rapidly to sulfonamide therapy, and later Thygeson and Stone (1942b) showed that topical applications of the sulfonamides were invariably successful in the treatment of the disease in the newborn

Clinical Picture

The incubation period of inclusion conjunctivitis varies from a minimum of 5 days to a maximum of 12. In the newborn the onset is acute, a purulent conjunctivitis developing rapidly with intense infiltration of the conjunctiva, particularly of the lower lid In very severe cases transient pseudomembranes are noted, and clinical differentiation from gonorrheal ophthalmia may be difficult After an acute stage, lasting from 10 days to 2 weeks, the disease gradually loses its intensity over a period of months The discharge may cease in as short a time as 2 months, but the conjunctiva rarely if ever returns to normal in less than 3 months and may show infiltration for as long as a year However, unlike trachoma, inclusion conjunctivitis never develops pannus or significant conjunctival cicatrization. It is always self-limited, and persistent chronic infections are unknown

The clinical appearance of the disease in the adult differs, often rather strikingly, from that in the newborn baby. Typically, it is an acute follicular conjunctivitis with scanty discharge and preauricular adenopathy; the follicular hypertrophy, unlike that of trachoma, is much more marked in the conjunctiva of the lower lid than in that of the upper. Occasionally, a severe infection may appear as a papillary conjunctivitis with moderately abundant exudate. In this form the adult dis-

ease more closely resembles the disease in the newborn but never exhibits the fulminating character so often seen in the latter. The adult disease tends to persist over a longer period of time, sometimes for more than a year. However, all reported cases have eventually resolved spontaneously without residual conjunctival or corneal changes.

Pathologic Picture

Examination of biopsy material from the disease in the newborn infant at onset shows an infiltration of the conjunctiva with small round cells, resulting in a many-fold increase in the thickness of the conjunctiva. The epithelium is infiltrated with polymorphonuclear cells and contains numerous basophilic cytoplasmic inclusion bodies morphologically identical with those of trachoma. The inclusion bodies are the first recognizable pathologic sign of the disease and have been demonstrated during the incubation period of experimental human infections. Biopsy material taken late in the course of the infection may show lymphoid follicles, a feature entirely lacking in the early stages.

In the adult the typical pathologic picture is a follicular hypertrophy of the conjunctiva associated with infiltration of small round cells. The follicles have the same histologic structure as those of trachoma but none of their necrotic features. Polymorphonuclear leukocytes predominate in conjunctival scrapings collected during the acute phase, but as the disease becomes chronic there is an admixture of mononuclear cells.

Experimental Infection, Host Range

Like trachoma virus, inclusion conjunctivitis virus infects only monkeys, baboons, apes and human beings. Baboons and apes are more susceptible than monkeys. Baboons inoculated with exudate develop an acute follicular conjunctivitis identical clinically with the adult human disease, but the clinical picture of inclusion blennorrhea in the newborn has never been reproduced in animals. Experimental inclusion conjunctivitis in lower animals is somewhat more intense than experimental trachoma. It is of shorter duration, and its inclusions are relatively easy to demonstrate. Otherwise, the two experimental diseases are indistinguishable. The relative intensity of experimental inclusion conjunctivitis reduces the likelihood of confusing it with spontaneous folliculosis. The cervix of the female baboon has been experimentally infected with inclusion conjunctivitis virus (Braley, 1939), but all attempts to induce experimental urethritis in the male baboon have been unsuccessful.

Etiology

The cause of inclusion conjunctivitis is a virus of large particle size morphologically identical with trachoma virus and showing the same intracellular cycle of morphologic development. No electronmicroscopic studies of it have as yet been made. It passes Berkefeld V candles, and its diameter, as determined by filtration through gradocol membranes, lies between 0.15 and 0.39 micron (Thygeson, 1934). It is destroyed rapidly by bile and by drying but can be preserved for several days in 50 per cent glycerol or at refrigerator temperature. All attempts to propagate it in chick embryo or human conjunctival tissue culture have failed. Group antibodies for the psittacosis-lymphogranuloma venereum viruses have been demonstrated in sera from patients with inclusion conjunctivitis (Rake et al., 1942).

The infectious units of inclusion conjunctivitis virus are elementary bodies with a diameter of about 0.25 micron in Giemsa-stained preparations. Masses of them can be seen as intracellular inclusion bodies (Fig. 117I), or free in the exudate in acute cases (Fig. 117H). Larger forms, first described by Lindner, 1910a, and known as initial bodies, are seen in initial-body inclusions and occasionally free in the exudate (Fig. 117J). They are apparently the forms produced when the virus first divides within the cytoplasm of susceptible cells. In the inclusion body the masses of elementary and initial bodies are embedded in a carbohydrate matrix identical with the matrix described for trachoma virus by Rice (Thygeson, 1938). According to observations made during the incubation period of experimental infections, the cycle of intracellular development requires about 48 hours. The inclusion bodies are limited strictly to the superficial layers of the epithelium (Braley, 1938). This applies also to the genito-urinary infection in the female (Fig. 117K). The existence of a toxin has been postulated to explain the subepithelial

changes that occur. The virus is not affected by sulfonamides in vitro, but the disease is rapidly cured by sulfonamide therapy. Morphologic observations indicate that the sulfonamide probably acts by preventing development of the virus in vivo rather than by killing it Topical applications of cortisone increase the susceptibility of the conjunctival and corneal cells to the virus and tend to reactivate quiescent cases.

Lindner suggested that trachoma virus and inclusion conjunctivitis virus were originally identical, that inclusion conjunctivitis virus, as a result of its sojourn on the mucous membranes of the genito-urinary tract through countless generations, eventually lost its ability to produce pannus and cicatrization, and that the two viruses bore a relationship to each other similar to that borne by the viruses of vaccinia and variola However, up to the present time no data to support this hypothesis have been submitted, and in 1944 Allen reported that inclusion conjunctivitis virus took on none of the characteristics of trachoma virus in the course of 40 serial transfers to the conjunctivas of human subjects. In this connection, Braley (1939) made the interesting observation that trachoma virus *could also produce infection of the cervix of* the female baboon

Diagnosis

The clinical diagnosis of inclusion conjunctivitis in the infant is facilitated by its delayed onset (5 to 12 days) and by the characteristic conjunctival thickening and coxcomblike appearance of the lower fornix *However, clinical diagnosis must be confirmed* by the finding of inclusion bodies Since trachoma never develops in newborn infants in the first week or two of life, the demonstration of inclusions in the infant is pathognomonic of inclusion conjunctivitis

In the adult an acute follicular conjunctivitis with mild preauricular adenopathy always suggests inclusion conjunctivitis. The absence of corneal changes is the clinical basis for differentiation from trachoma, but other forms of acute follicular conjunctivitis cannot always be distinguished clinically For the latter purpose laboratory diagnosis based on the finding of cytoplasmic inclusions is conclusive, except, of course, with respect to trachoma from which supplementary differentiation on clinical or cytologic grounds must be made. As noted above, the expressed follicular material from trachoma shows necrotic changes never seen in follicular material from inclusion conjunctivitis

The group antibodies for the psittacosis-lymphogranuloma venereum group of viruses that have been reported have no diagnostic significance in inclusion conjunctivitis

Inclusion conjunctivitis is the most important type of nongonococcal ophthalmia neonatorum, and clinical laboratories in all hospitals should be able to diagnose it, particularly in view of the serious social consequences which may result from confusing it with gonococcal ophthalmia For this purpose epithelial scrapings should be prepared instead of the exudate films used for determining bacterial infections.

Treatment

Prior to the introduction of the sulfonamides, no form of therapy had had any effect on inclusion conjunctivitis However, sulfonamide therapy is highly successful and may effect a clinical cure in as short a time as 5 days. Topical sulfonamide therapy, usually *employed 6 times* daily over a period of 10 days in the form of 5 per cent sulfacetamide or sulfadiazine ointment, has been uniformly successful in the newborn infant and irregularly successful in adults. Its lesser efficacy in adults is probably due to the dilution of the ointment by tears, a negligible factor in newborn infants whose tear-function is minimal in the first few weeks of life However, *oral administration* of the sulfonamides has been invariably successful in adult cases, and relapses almost never occur. No instance of a *sulfonamide-resistant* viral strain has as yet been reported. Secondary bacterial infection is not a problem.

Penicillin has not been effective in the treatment of inclusion conjunctivitis (Thygeson, 1947) but medium- and broad-spectrum antibiotics have been used successfully in ointment form.

Epidemiology

Eye-to-eye transmission of inclusion conjunctivitis is extremely rare, and no epidemics have been traced conclusively to ocular infec-

tion. The epidemiology is believed to parallel that of gonorrheal ophthalmia as it occurs in the United States and Europe The genito-urinary disease, like gonorrhea, serves as a reservoir from which infection of newborn eyes occurs during childbirth, and from which sporadic adult infections develop as a result of accidental transfer of genito-urinary exudate to the eye In all probability the eye disease would cease to exist if it were not for the genito-urinary reservoir of virus Doctors and nurses dealing with newborn infants and gynecologic conditions have been accidentally infected with inclusion conjunctivitis (Thygeson and Stone, 1942a), just as they have been accidentally infected with gonorrheal ophthalmia. Swimming-pool infection was a problem before chlorination was introduced, the virus being transmitted through the water from the genito-urinary tract to the eye, in fact, cases are still being reported from small lakes and unchlorinated pools Figure 117L shows the extraordinary number of virus elementary bodies that can be found in scrapings from the cervix and indicates what an abundant source of contamination the genito-urinary tract can be

The recent drop in the incidence of the disease may very well be due to the effect on the genito-urinary tract of the widespread use of sulfonamides and antibiotics in the treatment of other infections

Control Measures

Control measures parallel those applicable to the control of gonorrheal ophthalmia, except that the Credé silver-nitrate prophylaxis does not prevent inclusion blenorrhea The intracellular habitat of the virus no doubt serves to protect it from the action of the caustic Penicillin used as a substitute for silver nitrate has been no more effective Control of swimming-pool infection would seem to depend upon proper chlorination In a survey of university swimming-pools, all properly chlorinated, Thygeson and Stone (1924a) found no instance of infection This was in sharp contrast with the large number of cases known to have been contracted in unchlorinated pools.

Strict adherence to hand-washing precautions is sufficient to prevent transfer of the infection from a patient with the ocular disease to his attendants In nurseries, however, careful isolation should be enforced to prevent transfer from infant to infant

CLASSIFICATION

Although the agents of trachoma and inclusion conjunctivitis possess the essential properties of viruses (filterability, inclusion body formation and obligate cell parasitism), they are set apart from the typical large viruses, such as vaccinia virus and fowlpox virus, by a number of distinguishing properties These include their susceptibility to chemotherapy, the basophilic character of their inclusion bodies, and their intracellular cycle of morphologic variation

The similarity of the tinctorial and morphologic properties of the agents of trachoma and inclusion conjunctivitis to those of psittacosis virus was noted by Thygeson in 1934 and later stressed by Bedson and by Rake and Jones In 1938 Findlay, MacKenzie and MacCallum postulated an intracellular cycle of morphologic development for the elementary bodies of trachoma analogous to that described by Miyagawa and his associates for the elementary bodies of lymphogranuloma venereum Morphologically similar agents with the same tinctorial properties have since been isolated from mice, cats and calves

All these agents have the following important properties in common (1) they are relatively large and filter with difficulty, (2) they pass through an intracellular cycle of development from elementary body to initial body to elementary body, (3) they stain with simple basophilic dyes, (4) their colony form is a large, basophilic, cytoplasmic inclusion body, (5) they have common antigenic components that are demonstrable by the complement-fixation test, and (6) they are susceptible to certain antibiotics and sulfonamides Although differing among themselves in other respects and in their disease-producing potential, they would seem undeniably to form a transitional group between the typical large viruses and the rickettsiae

In 1945 Moshkovsky proposed the family name *Chlamydozoaceae* for the whole group, and the genus names *Miyagawanella* for the psittacosis-lymphogranuloma venereum agents, and *Chlamydozoon* for the agents of trachoma (*Chlamydozoon trachomatis*) and inclusion conjunctivitis (*Chlamydozoon oculogenitale*) This classification was accepted by Rake for inclusion in *Bergey's Manual of Determinative Bacteriology* in

1948 It has been protested by Meyer and Bedson but is widely employed at present, pending possible revision by an appropriate international taxonomic authority.

REFERENCES

(For complete references, see also 2nd edition, Chapter 21)

Allen, J H, 1944, Inclusion blennorrhea, Am J Ophth 27, 833-846

Bietti, G B, 1951, Memorandum on results obtained [illegible] treatment of trachoma by various antibiotics and by chemotherapy WHO/Trachoma/17, Sept 20

Bland, J O W, 1944, Spontaneous folliculosis of the conjunctiva in grivet and vervet monkeys, J Path & Bact 56, 161-171

———, 1945, The aetiology of trachoma, Brit J Ophth 29, 407-420

Braley, A [illegible], 1938, Inclusion blennorrhea a study of the pathologic changes in the conjunctiva and cervix, Am J Ophth 21, 1203-1207

———, 1939, Relation between the virus of trachoma and the virus of inclusion blennorrhea, Arch Ophth 22, 393-398

———, 1940, Intracellular bodies of the conjunctival epithelial cells, Arch Ophth 24, 681-689

Findlay, G M, Mackenzie, R D, and MacCallum, F O, 1938, A morphological study of the virus of lymphogranuloma inguinale (climatic bubo) 32, 183-188

Forster, W G, and McGibony, J R, 1944, Trachoma, Am J Ophth 27, 1107-1117

Halberstaedter, L, and von Prowazek, S, 1909, Ueber Chlamydozoenbefunde bei Blennorrhea neonatorum non gonorrhoica, Berl klin Wchnschr 46, 1839-1840

Julianelle, L A, 1937, Relation of inclusion blennorrhea to swimming-bath conjunctivitis as determined by an accidental transmission, Proc Soc Exper Biol & Med 36, 617-619

———, 1938, The Etiology of Trachoma, p 176, New York, Commonwealth Fund

Julianelle, L A, Harrison, [illegible] W, and Lange, A C, 1938, Studies on inclusion blennorrhea III Experimental considerations of the etiology Am J Ophth 21, 1230-1241

Julianelle, L A, and Smith, J E, 1942, Studies on the infectivity of trachoma XI The effect of sulfanilamide on the virus Am J Ophth 25, 317-321

[illegible] oma

[illegible] y su [illegible] illna,

[illegible] New York Acad Sc 56, 545

Michail, D, and Vancea, P, 1932, Quelques faits expérimentaux dans le trachome, Rev internat du Trachome 9, 33-36

Mitsui, Y, and Suzuki, A, 1956, Electron microscopy of trachoma virus in section, Arch Ophth 56, 429

Mitsui, Y, and Tanaka, C, 1951, Terramycin, aureomycin and chloramphenicol in the treatment of trachoma, Antibiotics & Chemother 1, 146-157

Mitsui, Y, Tanaka, C, Yamashita, [illegible], and Hanabusa, J, 1954, Toxin-like substance of trachoma virus, Arch Ophth 52, 72-76

Morax, V, 1933, Les conjonctivites folliculaires, Bull et mém Soc franc opht 46, 5-142

Moshkovsky, [illegible] D, 1945, The cytotropic agents of infections and the positions of rickettsiae in the system of chlamydozoa (in Russian), Uspekhi Souremennoi Biologii 19, 1-44

Poleff, L, 1949, Culture des corps du trachome à la lumière des acquisitions nouvelles, Rev internat du Trachome 26, 175-176

Rake, G, and Jones, H P, 1944, Studies on lymphogranuloma venereum II The association of specific toxins with agents of the lymphogranuloma-psittacosis group J Exper Med 79, 463-486

Rake, G, Shaffer, M F, and Thygeson, P, 1942, Relationship of agents of trachoma and inclusion conjunctivitis to those of lymphogranuloma-psittacosis group, Proc Soc Exper Biol & Med 49, 545-547

Rice, C E, 1936, The carbohydrate matrix of the epithelial-cell inclusion in trachoma, Am J Ophth 19, 1-8

Stewart, F H, and Badir, G, 1950, Experiments on the cultivation of trachoma virus in the chick embryo, J Path & Bact 62, 457-460

Thygeson, P, 1934, The etiology of inclusion blennorrhea, Am J Ophth 17, 1019-1035

———, 1938, The matrix of the epithelial cell inclusion body of trachoma, Am J Path 14, 455-462

———, 1939, Cultivation of trachomatous conjunctival epithelium in vitro, Arch Ophth 21, 229-234

———, 1941, Treatment of inclusion conjunctivitis with sulfanilamide, Arch Ophth 25, 217-226

———, 1946, The cytology of conjunctival exudates, Am J Ophth 29, 1499-1511

———, 1947, Unpublished observations

Thygeson, P, and Mengert, W F, 1936, The virus of inclusion conjunctivitis, Arch Ophth 15, 377-409

Thygeson, P, Proctor, F I, and Richards, P, 1935, Etiologic significance of the elementary body in trachoma, Am J Ophth 18, 811-813

Thygeson, P, and Stone, W, Jr, 1942a, Epidemiology of inclusion conjunctivitis, Arch Ophth 27, 91-119

———, 1942b, The treatment of inclusion conjunctivitis with sulfathiazole ointment, J.A.M.A 119, 407-408

Tilden, [illegible] B, and Gifford, S R, 1936, Filtration experiments with the virus of inclusion blennorrhea, Arch Ophth 16, 51-54

Wilson, [illegible] P, 1930, Folliculosis of the conjunctiva in animals, 4th Ann Rep Mem Ophth Lab (1929), Giza, Cairo, p 63

———, 1937, Inclusion bodies, 11th Ann Rep Mem Ophth Lab (1936), Giza, Cairo, [illegible] 117

———, 1945, A statement on the economic importance of the acute ophthalmias of Egypt, with recommendations for their control, 14th Ann Rep Mem Ophth. Lab (1939-44), Giza, Cairo, pp 38-48

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35

Measles

(SYNONYMS Morbilli, rubeola,* *rougéole,* Masern, sarampion)

INTRODUCTION

Measles is one of the important exanthemata The morbidity rate is very high, and by adult age, in denser centers of population, almost everyone has had the disease It is characterized by a prodromal period with fever, catarrh and cough, and exanthem in the mouth (Koplik's spots) Later there is a characteristic rash The prognosis, in the uncomplicated disease, is good, and even the complications, other than encephalitis, do well with modern chemotherapy

HISTORY

Measles was recognized as an independent disease by Sydenham in the 17th century, but the separation of it, perhaps with other exanthemata, was achieved by the Arabic physicians in the 9th century They considered it a milder form of smallpox It has appeared in epidemic form wherever man has established community life, and epidemics of particular severity have occurred when the virus has been introduced to a virgin soil, with the appearance of a very virulent strain (as in black measles in London, 1763-1768), or when accompanied by bacterial trailers (as in U S Army camps during World War I) Home in 1758 apparently transmitted the disease to 7 of 15 volunteers by placing cotton soaked with fresh blood of patients with florid disease in skin incisions of individuals with no previous history Hektoen, 1905, first achieved unequivocal transmission of measles to volunteers, after an incubation period of 11 to 13 days, by subcutaneous injection of blood taken within 30 hours after appearance of rash Anderson and Goldberger (1911) first transmitted measles to animals—macaque monkeys Plotz, 1938, cultivated the virus in chick cells, and Rake and Shaffer, 1939, described its cultivation in the embryonated chicken egg Enders and Peebles (1954) cultivated the virus in human post-nasal tissues and obtained typical cytopathogenic changes The prevention of the disease by inoculation of human serum containing neutralizing antibodies was first described by Nicolle and Conseil, 1918

CLINICAL PICTURE

The incubation period of the natural disease up to the appearance of the exanthem is from 12 to 19 days, usually exactly 14 days Following deliberate inoculation experiments, the incubation may be several days shorter, perhaps because the infecting doses used were larger than occur naturally However, the appearance of the typical exanthem is preceded by 1 to 5 days of prodromal symptoms, and the incubation period until the appearance of these is from 5 to 9 days

* Rubeola, unfortunately, is used as a synonym for both measles and rubella

1948. It has been protested by Meyer and Bedson but is widely employed at present, pending possible revision by an appropriate international taxonomic authority.

REFERENCES

(For complete references, see also 2nd edition, Chapter 21)

Allen, J H, 1944, Inclusion blennorrhea, Am J Ophth *27*, *833-846*

Bietti, G B, 1951, Memorandum on results obtained in treatment of trachoma by various antibiotics and by chemotherapy WHO/Trachoma/17, Sept 20

Bland, J O W., 1944, Spontaneous folliculosis of the conjunctiva in grivet and vervet monkeys, J Path & Bact *56*, 161-171

———, 1945, The aetiology of trachoma, Brit J Ophth *29*, 407-420.

Braley, A E, 1938, Inclusion blennorrhea a study of the pathologic changes in the conjunctiva and cervix, Am J Ophth *21*, 1203-1207

———, 1939, Relation between the virus of trachoma and the virus of inclusion blennorrhea, Arch Ophth *22*, 393-398

———, 1940, Intracellular bodies of the conjunctival epithelial cells, Arch Ophth *24*, 681-689

Findlay, G M, Mackenzie, R D, and MacCallum, F O, 1938, A morphological study of the virus of lymphogranuloma inguinale (climatic bubo) *32*, 183-188

Forster, W G, and McGibony, J R, 1944, Trachoma, Am J Ophth *27*, 1107-1117

Halberstaedter, L, and von Prowazek, S, 1909, Ueber Chlamydozoenbefunde bei Blennorrhea neonatorum non gonorrhoica, Berl klin Wchnschr *46*, 1839-1840

Julianelle, L A, 1937, Relation of inclusion blennorrhea to swimming-bath conjunctivitis as determined by an accidental transmission, Proc Soc Exper Biol & Med *36*, 617-619

———, 1938, The Etiology of Trachoma, p 176, New York, Commonwealth Fund

Julianelle, L A, Harrison, R W, and Lange, A C, 1938, Studies on inclusion blennorrhea III Experimental considerations of the etiology Am J Ophth *21*, 1230-1241

Julianelle, L A, and Smith, J E, 1942, Studies on the infectivity of trachoma XI The effect of sulfanilamide on the virus Am J Ophth *25*, 317-321

Loe, F, 1938, Sulfanilamide treatment of trachoma preliminary report, J.A.M.A *111*, 1371-1372

Macchiavello, A, 1944, El virus del tracoma y su cultivo en el saco vitelino del huevo de gallina, Rev. ecuator hig y med trop *1*, 211-243

Meyer, K F, 1953, Psittacosis group, Ann New York Acad Sc *56*, 545

Michail, D, and Vancea, P., 1932, Quelques faits expérimentaux dans le trachome, Rev internat du Trachome *9*, 33-36

Mitsui, Y., and Suzuki, A, 1956, Electron microscopy of trachoma virus in section, Arch Ophth *56*, 429

Mitsui, Y, and Tanaka, C, 1951, Terramycin, aureomycin and chloramphenicol in the treatment of trachoma, Antibiotics & Chemother. *1*, 146-157

Mitsui, Y, Tanaka, C, Yamashita, K, and Hanabusa, J., 1954, Toxin-like substance of trachoma virus, Arch Ophth *52*, *72-76*

Morax, V, 1933, Les conjonctivites folliculaires, Bull et mém Soc franc opht *46*, 5-142

Moshkovsky, S D, 1945, The cytotropic agents of infections and the positions of rickettsiae in the system of chlamydozoa (in Russian), Uspekhi Sourememnoi Biologii *19*, 1-44

Poleff, L, 1949, Culture des corps du trachome à la lumière des acquisitions nouvelles, Rev internat du Trachome *26*, 175-176.

Rake, G, and Jones, H P, 1944, Studies on lymphogranuloma venereum II The association of specific toxins with agents of the lymphogranuloma-psittacosis group J Exper Med *79*, 463-486

Rake, G, Shaffer, M F, and Thygeson, P, 1942, Relationship of agents of trachoma and inclusion conjunctivitis to those of lymphogranuloma-psittacosis group, Proc Soc Exper Biol & Med *49*, *545-547*

Rice, C E, 1936, The carbohydrate matrix of the epithelial-cell inclusion in trachoma, Am J Ophth *19*, 1-8

Stewart, F H, and Badir, G, 1950, Experiments on the cultivation of trachoma virus in the chick embryo, J Path & Bact *62*, 457-460

Thygeson, P, 1934, The etiology of inclusion blennorrhea, Am J Ophth *17*, 1019-1035

———, 1938, The matrix of the epithelial cell inclusion body of trachoma, Am J Path *14*, 455-*462*

———,
val ep

———,
with s

———, 1946, The cytology of conjunctival exudates, Am J Ophth *29*, 1499-1511

———, 1947, Unpublished observations

Thygeson, P, and Mengert, W F, 1936, The virus of inclusion conjunctivitis, Arch Ophth *15*, 377-409

Thygeson, P, Proctor, F I, and Richards, P, 1935, Etiologic significance of the elementary body in trachoma, Am J Ophth *18*, 811-813

Thygeson, P, and Stone, W, Jr, 1942a, Epidemiology of inclusion conjunctivitis, Arch Ophth *27*, 91-119

———, 1942b, The treatment of inclusion conjunctivitis with sulfathiazole ointment, J.A.M.A *119*, 407-408

Tilden, E H, and Gifford, S R, 1936, Filtration experiments with the virus of inclusion blennorrhea, Arch. Ophth *16*, 51-54

Wilson, R P., 1930, Folliculosis of the conjunctiva in *animals, 4th Ann Rep Mem* Ophth Lab (1929), Giza, Cairo, p 63

———, 1937, Inclusion bodies, 11th Ann Rep Mem Ophth Lab (1936), Giza, Cairo, p 117

——— ... on the economic importance ... om- ... Mem

Warthin and Finkeldey who described these cells independently in 1931 were not the first to note them. They were described by Ciaccio in 1910 and by Alagna in 1911 These cells occur as early as 5 days after exposure to infection (that is, before any symptoms) and they persist until after the rash has appeared They appear to arise from lymphocytes, but how is not clear.

Koplik's spots are focal exudations of serum and endothelial cells into submucous glands which form vesicles by necrosis of the basal epithelium The exanthem, according to the more recent work of Torres (1952), starts as a necrosis of a few epidermal cells Within 12 hours an exudation of serum occurs around the superficial vessels in the corium and proliferation of endoth...

...vesicles and later vesicopustules which dry up and desquamate Torres describes elongated acidophilic intranuclear inclusions in parakeratotic epithelial cells at the margin of the vesicles These are said to be surrounded by basophilic material and to be separated by clear zones from the nuclear membrane The recent observation of similar inclusions in tissue cultures of measles virus confirms Torres' observation In late stages of development of the exanthem the corium is edematous and infiltrated with monocytes containing keratohyalin granules By 72 hours the epidermal lesions have disappeared, but perivascular cuffs remain

In cases of encephalomyelitis the central nervous system shows, in the gross, congestion and petechial hemorrhages Microscopically, early cases show perivascular hemorrhage and lymphocytic infiltration, areas of demyelination later appear in brain and cord These areas are infiltrated with leukocytes and monocytes Finally, a gliosis appears

Cases of viral bronchopne...
acte
maci
lar ... appear in the
bronchial epithelium (Denton, 1925) In some cases the epithelial cells may be fused into large sheets which come to lie free in the bronchi The cell boundaries are lost, and each mass contains many nuclei which are pyknotic in the later stages On the denuded walls the epithelium rapidly regenerates, and squamous metaplasia may be seen Similar cell sheets have been described in the intestine

EXPERIMENTAL INFECTION; HOST RANGE

Josias, 1898, first indicated transmission of measles to monkeys, and successful transmission to *Macaca mulatta* (*Macacus rhesus*) was established by Anderson and Goldberger (1911) in the first unequivocal infection of any animal but man Besides *M mulatta*, *M. cynomolga*, *M sinica*, and *M fuscata* have been shown to be susceptible Monkeys can be infected ...

...man blood or catarrhal secretions (or the same from successfully infected monkeys) are used Infection can also be produced by inhalation and by contact Anderson and Goldberger (1911) found the latter very difficult to reproduce, but other considerations suggest that it may be easy when conditions are suitable Thus epidemics of measles have been seen in monkeys (Lapin, Director of the Med Biol Stat Acad Med Science Sukhund U S S R quoted by R E Shope in Account of observations by U S Med Miss to U S S R, 1955) Moreover, older monkeys kept in the laboratory have been known for years to be less susceptible to experimental infection with measles than are young and recently introduced animals Enders (1954) has been able to show that such lesser susceptibility in longer-held animals is associated ...

... varies with the dose of virus and route of inoculation used and may range from 3 to 22 days Viremia is established by the 4th or 5th day and lasts up to 15 days (Enders and Peebles, 1954) Various symptoms occur and differ in different animals The disease is rarely severe Exanthem is the most common sign Exanthem may be found in about 40 per cent of animals, conjunctivitis and catarrh, in 35 per cent, fever, in 30 per cent Mild symptoms suggestive of involvement of the nervous system have been observed occasionally Neutrophilopenia occurs in about 90 per cent of animals In severe infection all the above symptoms may occur, but usually only 2 or 3 appear in the same animal The disease may occur without any exanthem having been observed Virus can be

The prodromal symptoms are mostly catarrhal. *Onset may be sudden, with a chill followed* by sneezing, running nose, redness of the eyes, cough and fever A faint and scattered skin rash may occur, but the characteristic lesions of this early period are the Koplik or buccal spots which occur in 90 to 95 per cent of all cases These are usually bilateral and are grouped around the papilla of the parotid duct and on the mucous membrane of the lower lip. They measure from 1 to 3 mm. in diameter and are pale bluish-white in color set on a bright scarlet base They may be seen best by daylight. Similar lesions may occur in the conjunctivae and in the gastrointestinal tract, particularly in the colon

There is no interval between the prodromal stage and the fully developed disease Fever and cough become steadily worse for from 3 to 5 days, and then the rash appears, first on the forehead and behind the ears During the next 24 to 48 hours it spreads over face, neck, trunk and limbs. The fever falls after the rash is fully developed and is beginning to fade The exanthem is macular or maculopapular and may become confluent Hemorrhagic forms may occur, and when they do, the disease is severe Such hemorrhagic forms frequently show thrombocytopenia (Hudson et al, 1956) Formerly such severe cases were described as predominating in certain epidemics The rash disappears on pressure. After it fades it leaves a brownish staining which is followed by branny desquamation There may be frank conjunctivitis with photophobia Leukopenia is usual at the height of the infection.

Virus is present in the blood and in the nasopharyngeal secretions, probably during the whole prodromal period and up to 30 hours following the appearance of the rash

Bacterial complications such as otitis media, with perforation of the drum, and bronchopneumonia are not infrequent, but with modern chemotherapy are rarely serious The pneumonia may be purely bacterial and due to streptococci, pneumococci or *H. influenzae.* It was in such cases formerly that the high mortality occurred. A pure viral type of pneumonia has also been described (Moore and McCordock, 1934; Corbett, 1945; Milles, 1945). In the roentgenogram the picture resembles primary atypical pneumonia; sometimes in addition miliary nodules are observed

Encephalomyelitis is the most serious complication today. Fortunately, it is rare (about 1 in 10,000 cases), but in certain epidemics the incidence may be higher It shows a definite tendency to be associated with the more severe cases of the disease, is more frequent in girls than in boys and in the white race than in the colored It may develop before the disappearance of the exanthem and the usual period of fever, but usually there have been several days of return to normal when, abruptly, the temperature begins to rise again, and the patient becomes drowsy or hyperexcitable with convulsions Headache, vomiting and coma occur Protein and cells are increased in the spinal fluid The mortality is from 10 to 16 per cent but rises to 38 per cent in those patients who show coma. A proportion of those who survive show permanent mental and physical changes The incidence of such sequelae is stated differently by various observers: from 15 per cent by Hodes (personal communication) to 65 per cent by Ford, 1928 Besides encephalomyelitis other forms of involvement of the central nervous system occur but more rarely. These are myelitis, radiculitis or neuroretinitis.

It has been pointed out above that lesions comparable with Koplik spots may occur in the gastro-intestinal tract The appendix may be involved, and either these lesions or the hyperplasia of the lymphoid tissue can lead to symptoms of appendicitis Appendectomies because of faulty diagnosis are not unknown

Goldfield et al (1955) have described changes in the electrocardiograms of 19 per cent of patients with measles The changes were transient but could not be attributed to occurrence of fever, complications or disturbances of acid-base balance

PATHOLOGIC PICTURE

The catarrhal inflammation is not typical ... the body may be

pendix. The cells (often called Warthin-Finkeldey cells from their presumed original observers) measure up to 100 μ across and may contain as many as 100 nuclei Actually,

disease in cynomolgus monkeys Viremia could be demonstrated 4 to 5 days after intravenous or intranasal inoculation and persists for 2 to 5 days (Enders et al, 1957) The disease picture has been described above. Complement-fixing antibodies appear on about the 14th day, diminish in titer after several weeks, but are still detectable at least 8 months after inoculation As indicated above, it appears that monkeys become spontaneously infected with measles in the laboratory, and isolation of apparently typical measles virus from monkey kidneys has been described by Ruckle (1956) by Frankel et al (1957), and by Muething et al (1957) Using a strain of measles virus which had been modified by continued passage in human amnion tissue culture and in which the refractile stellate cell had become predominant (see above), Enders et al (1957) were able to obtain successful transmission in the chick embryo by the amniotic sac By passing at 9-day intervals, the strain was maintained in the chick embryo for 7 passages The chick virus was neutralized by convalescent phase human and monkey sera Attempts to transmit strains of earlier tissue culture passage to the chick embryo were not successful Katz et al (1958) have also been able to grow a strain of measles virus already well established in the laboratory in cultures of chick amniotic membrane For the first 4 passages no cytopathic changes were produced, but thereafter they occurred regularly

Adams and Imagawa (1957) have drawn attention to a most interesting antigenic relationship between the virus of measles and that of canine distemper Thus the characteristic cytopathic changes of measles virus in tissue culture can be neutralized by specific distemper antiserum, ferrets immunized with measles virus show modified disease when challenged with virulent measles virus, and distemper in the suckling mouse can be neutralized with convalescent human antimeasles serum or serum from ferrets immunized with measles virus These interesting observations merit much further attention It is particularly striking to be presented with such evidence of serologic relationship for these two viruses whose similar biologic behavior in their respective hosts has been apparent to students of measles or distemper for years

DIAGNOSIS

During an epidemic diagnosis is easy The enanthem (Koplik's spots) rather than the rash is diagnostic of sporadic cases Tompkins and Macaulay (1955) have been able to demonstrate the Alagna (Warthin-Finkeldey) multinucleate giant cells in the sputum and the nasal mucus during the prodromal stage and up to the appearance of the exanthem This may well prove to be a simple and useful diagnostic procedure In 5 per cent of cases Koplik s spots may not be observed, and differentiation of mild cases from rubella may be difficult In rubella early and marked adenitis is characteristic Severe forms of catarrhal laryngitis preceding the measles rash must be distinguished from that of scarlet fever, rubella and smallpox The rash of infectious mononucleosis or that of dengue may be morbilliform, as may allergic rashes due to drugs The Debre test may also be used in diagnosis In this test intradermal injection of measles convalescent serum will prevent appearance of measles rash specifically in the area infiltrated

TREATMENT

In uncomplicated cases treatment is largely symptomatic Bed rest and light diet are indicated along with subdued light because of the conjunctivitis and photophobia A sedative or antitussive may be required for the cough Secondary bacterial infections can be combated successfully with sulfonamides or antibiotics The use of these drugs prophylactically is open to question Weinstein (1955) presents data indicating that secondary bacterial complications, especially with *H influenzae*, occur twice as often when antibiotics have been used in attempted prophylaxis Therefore, chemotherapy should not be employed until there is definite evidence of bacterial complication

Specific therapy consists of the use of serum antibodies prepared from convalescent serum, pooled adult serum or placental extracts. The use of any of these depends on the fact that most adults have had measles and therefore collection of adult blood will contain large amounts of specific antimeasles gamma globulin More recently, concentrates of fractionated gamma globulin, containing mostly

demonstrated in the buccal mucosa and secretions and in the blood of infected monkeys The pathologic picture of the enanthem and the exanthem in monkeys is essentially similar to that in man. No mammals other than primates have been successfully infected with measles

ETIOLOGY

The virus in blood or nasopharyngeal secretions passes through Berkefeld N or Seitz EK filters It can be preserved at −72° C or −35° C. for periods up to 4 weeks and for several days at 0° C At room temperature infectivity is retained for 1½ days It may be dried in vacuo from the frozen state and then will remain active for at least 15 weeks At −72° C. it resists a pH of 6 for 60 hours, but lower pH ranges rapidly inactivate it It withstands 10 per cent ether for 40 minutes at room temperature

Many claims of successful cultivation of the virus of measles have been made and are reviewed by Rake et al. (1941) on studies with tissue culture, and by Shaffer et al (1941) on studies with chicken embryos In many cases the agents cultivated would seem not to be those responsible for measles The first successful cultivation of measles seems to have been that of Plotz in chick embryo tissue in 1938, and Rake and Shaffer in 1939 demonstrated transmission of measles to monkeys with virus grown in chicken embryos after inoculation on the chorio-allantois by the Burnet technic, or into the amniotic or the allantoic cavities This work was complicated by the fact that no alteration was observed in the tissues, either in the gross or microscopically Such egg-passaged virus also was capable of producing disease in man which was but slightly modified in early passages but became more modified with subsequent passage

In 1954 Enders and Peebles described the cultivation, in human postnasal tissue growing in roller tubes, of a filterable agent from the blood and throat washings taken within the first 24 hours from a patient with measles Cytologic changes appeared in from 4 to 10 days. Subsequently, a similar agent was grown in human or rhesus renal cells again from blood or throat washings, or in 1 case from lung Similar viruses have been obtained by Cohen et al. (1955) and by Frankel et al (1957). The latter also isolated the virus from conjunctiva and from stools 4 to 5 days after appearance of the rash and reported successful primary isolation in both monkey kidney cells and in human amnion. Enders et al (1957) have described in detail the changes appearing in the tissue cells In human renal cells multinucleated giant cells appear These cells show intranuclear acidophilic inclusion bodies and masses of eosinophilic material in the cytoplasm Similar giant cells have been observed in human amnion, in various lines of human malignant epithelial cells and in cultures of human fibroblasts No cytopathogenic effect has been seen in bovine or chick tissues tested After continued passage the cytopathic effects change and, besides the giant cells, increasing numbers of refractile stellate cells appear. In time these latter cells may be the only ones observed They also show intranuclear inclusions Studies of distribution of measles viral antigen within the cells have been made by Cohen et al. (1955) and by Baker et al (1957) using the fluorescent antibody technic of Coons and Kaplan Fluorescence was seen by Cohen et al by the 5th day in the cytoplasm and later as masses in the nucleus It increased in amount with tissue culture passage Baker et al found fluorescent antigen combination as early as the 4th day Fluorescence could be induced only with measles convalescent serum. The technic was more sensitive than the complement-fixation test in detecting antimeasles antibody. Enders et al. (1957) describe the development of both neutralization tests—testing the capacity of sera to prevent the cytopathogenic effects of the virus—and complement fixation, using supernatant fluid from tissue cultures of infected human renal or amnion cells as antigen By both technics it can be shown that acute phase sera show no antibodies but that an increase appears soon after the rash appears and reaches titers as high as 1:512. These high initial titers are maintained for up to 4 months (longest tested) but presumably will decrease after that time, since sera from patients with past history of measles show, in average, lower titers.

Tissue-culture measles virus produces mild

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Tissue-culture measles virus produces mild

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REFERENCES

(For complete references, see also 2nd edition of this book, Chapter 22)

Adams, J J, and Imagawa, D T, 1957, Immunological relationship between measles and distemper virus, Proc Soc Exper Biol & Med 96, 240 244

Alagna, G, 1911, Histopathologische Veranderungen der Tonsille und der Schleimhaut der ersten Luftwege bei Masern, Arch Laryng Rhin (Berl) 25, 527-530

Anderson, J F, and Goldberger, J, 1911, Experimental measles in the monkey a preliminary note, Pub Health Rep 26, 847-848

Baker, R F, Rapp, F, Grogan, E A, and Gordon, I, 1957, Visualization of measles virus in human cells, Bact Proc, p 76

Ciaccio, C C, 1910, Beitrag zur pathologischen Anatomie und zur Mikrobiologie der Masern, Arch path Anat 199, 378-400

Cohen, S M, Gordon, I, Rapp, F, Macaulay, J C, and Buckley, S M, 1955, Fluorescent antibody and complement-fixation tests of agents isolated in tissue culture from measles patients, Proc Soc Exper Biol & Med 90, 118-122.

Corbett E V, 1945, The visceral lesions in measles, Am J Path 21, 905-914

Denton, J, 1925, The pathology of fatal measles, Am J M Sc 169, 531-543

Enders, J F, 1954, Recent observations on the behavior in tissue culture of certain viruses pathogenic for man Fourth R E Dyer Lecture Pub Health Serv Pub No 467 Washington, D C

Enders, J F, and Peebles, T C, 1954, Propagation in tissue cultures of cytopathogenic agents from patients with measles, Proc Soc Exper Biol & Med 86, 277-286

Enders, J F, Peebles, T C, McCarthy, K, Milovanovic, M, Mitus, A, and Holloway, A, 1957, Measles virus a summary of experiments concerned with isolation properties and behavior, Am J Pub Health 47, 275 282

Frankel, J W, Burnstein, T, Devaney, J T, and West, M K, 1957, Studies on isolates of measles virus, Bact Proc, p 76

Goldfield, M, Boyer, N H, and Weinstein, L, 1955, Electrocardiographic changes during the course of measles, J Pediat 46, 30-35

Greenberg, M, Appelbaum, E A, Pellitteri, O, and Eisenstein, D T, 1955b, Measles encephalitis II Treatment with γ globulin, J Pediat 46, 648-653

Greenberg, M, Pellitteri, O, and Eisenstein, D T, 1955a, Measles encephalitis. I Prophylactic effect of γ globulin, J Pediat 46, 642-647

Hudson, J B, Weinstein, L, Chang, F W, 1956, Thrombocytopenic purpura in measles, J Pediat 48, 48-56

Katz, S L, Milovanovic, M V, and Enders, J F, 1958, Propagation of measles virus in cultures of thick embryo cells. Proc Soc Exper Biol & Med 97, 23-29

Milles, G, 1945, Measles pneumonia, Am J Clin Path 15, 334-338

Moore, E, and McCordock, H A, 1934, Encephalomyelitis and "hemorrhagic virus pneumonia" complicating measles, Arch Neurol & Psychiat 32, 560-565

Muething, D, Patch, J, McClean, I W, Jr, and Rightsel, W A, 1957, Propagation and characteristics of measles virus in tissue culture, Bact Proc, p 76

Rake, G Shaffer, M F, and Jones, H P, 1941, Studies on Measles III The use of tissue culture in propagation of measles virus J Infect Dis 69, 65-69

Ruckle, G, 1956, Measles in humans and monkeys report of isolation from cynomolgus monkeys of an agent immunologically related to human measles virus, Fed Proc 15, 610-611

Shaffer, M F, Rake, G, Stokes, J, Jr and O'Neil, G C, 1941, Studies on measles II Experimental disease in man and monkey J Immunol 41, 241-257

Stillerman, M, Marks, H H, and Thalhimer, W, 1944, Prophylaxis of measles with convalescent serum, Am J Dis Child 67, 1-14

Stokes, J, Jr, Maris, E P, and Gellis, S S, 1944, Chemical, clinical and immunological studies on the products of human plasma fractionation XI The use of concentrated normal human serum gamma globulin (human immune serum globulin) in the prophylaxis and treatment of measles J Clin Invest 23, 531-540

Tompkins, V, and Macaulay, J C, 1955 A characteristic cell in nasal secretions during prodromal measles, J.A.M.A 157, 711

Torres, C M, 1952, Alterações microscópicas na erupção do Sarampo, Mem Inst Oswaldo Cruz 50, 1

Weinstein, L, 1955 Failure of chemotherapy to prevent the bacterial complications of measles, New England J Med 253, 679-683

gamma but also some beta globulin, have been used. Before the availability of the globulin concentrates therapy was administered, almost entirely, during the incubation period when exposure was known to have occurred, to prevent or modify the disease Complete suppression is advisable in the case of very young or sick children and is obtained by use of a large dose of whatever preparation is employed, sufficiently early after exposure In children 3 years or younger this would amount to 5 cc of convalescent or 10 cc. of adult serum within 6 days after exposure. For older children, the age is multiplied by 2 for convalescent serum or by 4 for adult serum to obtain the dose in cubic centimeters For attenuation a smaller dose is used, or the injection is given later after exposure Thus the same doses may be used as for prevention but given later than 6 days after known exposure, or half the amount may be given on about the third day. In the case of globulin concentrates, 0 5 cc. given to children under 6 years within 7 days of exposure results in complete protection of about half, and modified disease in the others If with any of the preparations an ideal dose is achieved, the disease may be modified to a point where no true malaise occurs and yet the individual is protected against any subsequent exposure. Stillerman et al. (1944) have pointed out the frequency with which very mild symptoms are seen by the careful observer following doses presumed to be preventive Without careful examination such symptoms are usually entirely overlooked However, if too large a dose is used, resulting in no symptoms or markedly modified ones, the protection produced will be at best only temporary The failure of markedly modified experimental disease to produce immunity has also been observed.

Although the above treatments are often

highly concentrated preparations of immune globulin, showing antibody titers 15 to 35 times as high as the original plasma pool, attempts have been made to influence the course of the disease with injections given as late as the prodromal stage or even later Stokes et al (1944) believe that the incidence of encephalitis can be affected, but Greenberg et al. (1955b) could obtain no such evidence, although encephalitis was apparently less frequent in individuals in whom early modifying doses of gamma globulin had been given (Greenberg et al. 1955a) Apart from encephalitis the prognosis of the disease is good today.

EPIDEMIOLOGY

Measles is one of the most infective of the exanthemata It is particularly infective during the catarrhal prodromal stage when the

with secretions can carry the disease for a short period, as can on rare occasions indi-

patients

wanes

isles is

endemic in all populous regions and occurs in epidemic form at approximately 3-year intervals. Only 1 per cent of susceptible individuals fails to contract the disease on their first close contact with a case in the infective stage By the age of 20 some 90 per cent of persons in civilized countries have had the

attacks are unusual but may occur, and certain rare individuals appear never to acquire an immunity and suffer repeated typical attacks of the disease

CONTROL MEASURES

Early reporting of cases of measles is important. When the disease is recognized in a community any known susceptible child with catarrhal symptoms is suspect, particularly if there is history of exposure, and the appearance of enanthem should be watched for. Isolation of the patient and disinfection of articles soiled with catarrhal secretions should be carried out from the first appearance of the prodromal symptoms until 4 to 5 days after appearance of the rash—a period of some 9 to 10 days in all Quarantine of children during epidemics in large communities is of little value. Quarantine of exposed individuals for 14 days in sparsely settled communities may be practiced, at least to the extent of forbidding such individuals to attend school or public gatherings Segregation of infants,

and encephalitis, are more frequent in adults than in children. When encephalitis occurs it usually appears 3 or 4 days after the appearance of the rash (Mitchell and Pampiglione, 1954). Headache and apathy may be followed rapidly by coma. The incidence of encephalitis after rubella is definitely less than after measles. The mortality is 20 per cent (Miller, 1956), but if death does not occur, despite evidence of widespread involvement of the central nervous system, complete though slow recovery is the rule. Gross mental disturbance may occur.

Gregg (1941) first showed the serious effects on the fetus of rubella occurring in the mother in the early months of pregnancy. Attention was drawn to cataract and cardiac malformations. Subsequent studies in Australia and later in the United States, Britain and Europe have confirmed the original observations and have shown that the effects on the fetus are not due to any particularly severe strain of the virus. Attention should be drawn to 3 surveys in Australia of the results of rubella during pregnancy (Gregg et al., 1945, Swan et al., 1946, Swan 1949). From these it appears that rubella produces serious congenital malformations only when it occurs during the first 4 months of pregnancy. The rate may vary from 83 per cent if infection of the mother occurs during the first postconceptual month to 61 per cent in the 4th month. American statistics (Ingalls and Gordon, 1947, Ober et al. 1947) suggest a lower defect rate—50 per cent following rubella in the first trimester. However, it should be pointed out that the American statistics were collected at an earlier date after birth than were the Australian, and many of the commoner abnormalities, particularly deafness, would not be included. Although of less frequency than the abnormalities in the [illegible]

that the most frequent defect is deafness with secondary mutism (75 per cent). *Microcephaly occurs in 70 per cent; eye defects,* particularly cataract, in 66 per cent, and cardiac malformations in 63 per cent. Dental abnormalities, often of major character, appeared in 45 per cent. In a more recent communication Greenberg et al. (1957) suggest that actually the incidence of defects may be considerably lower than indicated by all other surveys, i.e., not higher than 12 per cent. These workers believe that other surveys suffer by being "retrospective" rather than "prospective." However, it should be pointed out that since the data in this study were obtained during the first year after birth, defects in dentition and some deficiencies in hearing would not have been detected.

PATHOLOGIC PICTURE

Although the lymph nodes are swollen, and some authors note enlargement of the spleen, [illegible]

A small increase in protein and a large increase in number of lymphocytes occur in the spinal fluid. [illegible]

patent ductus arteriosus and foramen ovale. Swan (1944) notes that no sign of endarteritis obliterans could be found in the ducti arteriosi. The children were usually undernourished, with indications that all cells were involved to some degree. Affected eyes were small (microphthalmia), and the lens showed massive central or nuclear necrosis with distortion of the peripheral lens fibers. Carruthers, 1945, examined the ears in one case with deafness and found absence of differentiation of primitive cells to form the organ of *Corti*.

EXPERIMENTAL INFECTION, HOST RANGE

The disease may be transmitted to susceptible children by the inoculation of material containing active virus. Virus may be found in the blood in the natural disease and also in the nasopharyngeal secretions from the time of the appearance of the prodromal symptoms up to 30 hours after the appearance of rash. As Hiro and Tasaka, 1938, showed in their original observation, not all children showed typical infection, and some

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36

Rubella

(SYNONYMS German Measles, rubeola,* epidemic roseola, 3-day measles, *Rotheln* or *Roteln, rubéole*)

INTRODUCTION

Rubella is one of the frequent mild exanthemata, usually occurring in childhood It is now recognized that the disease may produce serious effects in the fetus when it is acquired by the mother at certain stages of pregnancy, but in the mother herself, the disease, if somewhat severer than occurs in childhood, is not serious. Apart from effects in the unborn child, the prognosis is usually excellent

HISTORY

Although rubella undoubtedly has existed for many centuries, confusion of it with other exanthemata has obscured its true identity until comparatively recently. Wagner, 1829, first separated it from measles and scarlet fever, but his observation was largely ignored for the next 50 years Confusion between rubella and measles still exists, and probably

on the oral mucosa in measles but not in rubella. Until comparatively recently rubella has been considered a mild and unimportant disease, but this view has been altered by the observation of Gregg (1941) and others, on the serious effect of the disease in pregnant females on the fetus in utero Recently (1954), Anderson has demonstrated the cultivation of the agent in tissue culture

CLINICAL PICTURE

The symptoms are usually mild, particularly in children The incubation period is usually 18 days, with a range from 14 to 23 The disease begins with a short prodromal peroid of mild catarrhal symptoms, fever and malaise but with no Koplik's spots When the rash appears it starts on the head and the face and spreads within 24 hours to the neck and the trunk In the early stages it resembles the rash of measles, since it consists of round, pink and slightly raised macules, usually discrete. If confluent, it resembles the rash of scarlet fever; rarely, it may be papular. The exanthem lasts only 2 or 3 days and characteristically may appear on new areas after it has faded from those first affected. Slight branny desquamation may occur Although Koplik's spots do not occur, a fine red enanthem may appear on the soft palate, and the tonsils may be enlarged. Fever is usually slight and continues while the rash persists Lymphadenitis is characteristic and affects particularly the cervical and the occipital nodes, which may be tender. The swelling may persist for 2 or 3 weeks. Complications are rare, and those that occur, such as arthritis, neuritis

* Rubeola, unfortunately, has been used as a synonym for both measles and rubella

characteristic. A rubellalike rash may be produced by allergy to drugs.

TREATMENT

The treatment of the disease is usually symptomatic, and in many cases the disease is so mild that not even rest in bed is required. The demonstration of the frequent and serious effects produced in the fetus by rubella acquired by the mother during the first 4 months of pregnancy have changed ideas as to the gravity with which this infection should be viewed If a pregnant woman is exposed to rubella, prevention of the infection may be attempted by the use of immune globulin in one form or another (Chap 34). However, the value of such prophylaxis is debatable, and certainly the results are not consistent Barenberg et al (1942) present indication of some suppressive action, and McLorinan (1950) showed that 4 ml. of gamma globulin from patients convalescent from rubella will prevent disease in the majority of susceptible individuals if given not later than the 8th day after contact More recently, Korns (1952) has shown that certain lots of serum globulin are effective It is possible that the use of serum or gamma globulin to protect the mother during pregnancy may also prevent malformations in the fetus

Because unfortunately the effect of convalescent serum or globulin is not entirely certain, the problem remains as to how to handle the case of a woman exposed to and later developing rubella during the first 3 or 4 months of pregnancy Many factors have to be taken into consideration, including parity and religion When rubella develops early in pregnancy in a recently married, healthy young woman, the situation should be explained in detail to the couple, and therapeutic abortion advised In the case of an older childless couple, the pregnancy should be allowed to go to term, and a calculated risk taken of the consequences of rubella to the fetus

EPIDEMIOLOGY

Rubella occurs in epidemics most frequently in the winter and spring months Incubation is from 14 to 21 days—usually 18 An attack is almost always followed by permanent immunity Diagnosis of second attacks of rubella are usually due to confusion with other mild exanthemata, as has been discussed elsewhere. The disease is transmitted primarily by virus-laden discharges from the nasopharynx of the patient in the prodromal period, or during the first 24 hours after appearance of rash Transmission usually occurs directly but may also occur by means of articles freshly soiled with infected secretions. The contagiousness is less than in measles, as is indicated by the higher attack rate for rubella in adults

CONTROL MEASURES

Before the serious results of an attack of rubella early in pregnancy were recognized, control measures were deemed of little importance It is now clear that every attempt should be made to assure that girls contract rubella before the child-bearing age If a pregnant woman has not had rubella, or even if she has (because of possible faulty diagnosis of the earlier attack), every attempt should be made to isolate her from cases of the disease during the first 4 months of pregnancy. If, despite precautions, she is exposed, especially if she is older and thus far childless, prophylaxis with immune globulin should be attempted for lack of anything better A definitive study of conditions under which such globulin may be effective is urgently required

REFERENCES

(For complete references, see also 2nd edition of this book, chapter 23)

Anderson, S G, 1949, Experimental rubella in human volunteers, J Immunol *62*, 29-40

———, 1954, Rubella virus in tissue culture, Lancet *2*, 1107-1108

Barenberg, L H, Levy, W, Greenstein, N M, and Greenberg, B, 1942, Prophylactic use of human serum against contagion in a pediatric ward, Am J Dis Child *63*, 1101-1109

Gregg, N M., 1941, Congenital cataract following German measles in the mother, Tr Ophth Soc Austral (M M A) *3*, 35 46

Gregg, N M, Beavis, W R., Heseltine, M, Machin, A E, Vickery, D, and Meyers, E., 1945, The occurrence of congenital defects in children following maternal rubella during pregnancy, M J Australia, *2*, 122-126

Greenberg, M, Pellitteri, O, and Barton, J, 1957, Frequency of defects in infants whose mothers had rubella during pregnancy, J.A.M.A *165*, 675-678

may have the disease without any sign of rash Anderson (1949) was able to infect volunteers with a spray of freshly prepared throat washings taken from patients with rubella during the first 24 hours after appearance of rash. In these cases the incubation period varied from 13 to 20 days, and lymphadenopathy might precede the rash by as long as 6 days. Rubella could be transmitted naturally to susceptible contacts exposed to these children during their actual disease. Some volunteers apparently had subclinical infections. After injection of material, the incubation period varies from 5 to 7 days Krugman et al (1953) were also able to produce infection in volunteers by intramuscular or intranasal inoculation of blood or nasopharyngeal washings taken from patients 13 hours after the appearance of their rash In this case the incubation period was from 12 to 16 days, regardless of the route of inoculation Contact infection by exposure to the inoculation disease occurred, and some of the inoculated volunteers showed what was apparently *rubella sine eruptione*.

Hess, 1914, was the first to demonstrate possible successful transmission of rubella to monkeys. He used *M. mulatta* and inoculated them with blood taken from patients within 24 hours of the appearance of the rash One animal showed a sharp febrile response in the 19th day. Habel (1942) also used *M mulatta* (rhesus monkeys). Blood or nasopharyngeal washings, collected within 12 hours of appearance of rash, were used In 13 out of 16 attempts mild disease was produced following subcutaneous, intraperitoneal, intranasal or intravenous inoculation of filtered or unfiltered material. Incubation was from 7 to 9 days Pyrexia was slight, as was leukopenia. The exanthem, which was not always present, consisted of sparse pink macules, chiefly on the trunk. The disease was passed from monkey to monkey for 5 passages The disease did not in every case protect against challenge inoculation 1 to 3 months later Krugman et al. (1953) were unable to infect cynomolgus monkeys

ETIOLOGY

The successful transfer of rubella to children accomplished by Hiro and Tasaka, 1938, and discussed above, was with nasal washings filtered through Berkefeld W or Seitz EK filters. Habel (1942) was able to obtain successful transmission after passage through Berkefeld N filters, and Anderson (1949) obtained passage of the agent through a filter with pore diameters of 800 μ. The virus remains viable after 3 months at $-70°$ C. or after 9 months storage in a dry-ice chest

Habel (1942) described the passage of rubella on the chorio-allantois of the chicken embryo No lesions developed, but successful transmission to the monkey was noted after 5 such passages. This work has not yet been confirmed

Anderson (1954) was able to cultivate an agent in monkey kidney cells in roller tubes from cases of rubella After 5 passages there appeared on the 5th day, groups of multinucleated cells. The cytopathogenic agent could be passed subsequently to human fetal lung and other fetal tissues. The cytopathogenic changes were neutralized by rubella convalescent serum but not by measles convalescent serum.

DIAGNOSIS

Rubella must be distinguished from measles, exanthem subitum, erythema infectiosum, infectious mononucleosis, and from dengue in those areas where the latter disease occurs. Differential diagnosis from the first is best established by the absence of Koplik spots, but the milder course of rubella, the enlarged cervical nodes and the distribution of the exanthem are useful features Exanthem subitum starts abruptly with fever, and rash is unusual before the fever terminates; the disease is even milder than rubella and rarely occurs in other than infants and young children. In erythema infectiosum the rash is characteristic, and the patient rarely shows any other sign or symptom—not even fever or malaise The rash in infectious mononucleosis is inconstantly present and protean in type. It may mimic any of the common exanthemata. Usually, however, there is a marked rise in the count of primitve monocytes and lymphocytes. Heterophile antibodies are found In dengue, the abrupt and high fever and the severe pain in muscles and joints are

characteristic. A rubellalike rash may be produced by allergy to drugs.

TREATMENT

The treatment of the disease is usually symptomatic, and in many cases the disease is so mild that not even rest in bed is required The demonstration of the frequent and serious effects produced in the fetus by rubella acquired by the mother during the first 4 months of pregnancy have changed ideas as to the gravity with which this infection should be viewed. If a pregnant woman is exposed to rubella, prevention of the infection may be attempted by the use of immune globulin in one form or another (Chap 34) However, the value of such prophylaxis is debatable, and certainly the results are not consistent Barenberg et al (1942) present indication of some suppressive action, and McLorinan (1950) showed that 4 ml. of gamma globulin from patients convalescent from rubella will prevent disease in the majority of susceptible individuals if given not later than the 8th day after contact More recently, Korns (1952) has shown that certain lots of serum globulin are effective. It is possible that the use of serum or gamma globulin to protect the mother during pregnancy may also prevent malformations in the fetus

Because unfortunately the effect of convalescent serum or globulin is not entirely certain, the problem remains as to how to handle the case of a woman exposed to and later developing rubella during the first 3 or 4 months of pregnancy Many factors have to be taken into consideration, including parity and religion When rubella develops early in pregnancy in a recently married, healthy young woman, the situation should be explained in detail to the couple, and therapeutic abortion advised In the case of an older childless couple, the pregnancy should be allowed to go to term, and a calculated risk taken of the consequences of rubella to the fetus

EPIDEMIOLOGY

Rubella occurs in epidemics most frequently in the winter and spring months. Incubation is from 14 to 21 days—usually 18 An attack is almost always followed by permanent im- [illegible]

[illegible]

patient [illegible]
the first 24 hours after appearance of rash [illegible]

CONTROL MEASURES

Before the serious results of an attack of rubella early in pregnancy were recognized, control measures were deemed of little importance It is now clear that every attempt should be made to assure that girls contract rubella before the child-bearing age If a pregnant woman has not had rubella, or even if she has (because of possible faulty diagnosis of the earlier attack), every attempt should be made to isolate her from cases of the disease during the first 4 months of pregnancy. If, despite precautions, she is exposed, especially if she is older and thus far childless, prophylaxis with immune globulin should be attempted for lack of anything better. A definitive study of conditions under which such globulin may be effective is urgently required

REFERENCES

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Anderson, S G , 1949, Experimental rubella in human volunteers, J Immunol 62, 29-40

———, 1954, Rubella virus in tissue culture, Lancet 2 1107-1108

B[illegible] N M and [illegible]

Gregg, N M , 1941, Congenital [illegible] German measles in the mother, Tr Ophth Soc Austral (B M A) 3, 35-46

Gregg, N M , Beavis, W R., Heseltine, M , Machin, A E , Vickery, D , and Meyers, E , 1945, The occurrence of congenital defects in children following maternal rubella during pregnancy, M J Australia, 2, 122-126

Greenberg, M , Pellitteri, O , and Barton, J , 1957, Frequency of defects in infants whose mothers had rubella during pregnancy, J A M A 165, 675-678

Habel, K , 1942, Transmission of rubella to *Macacus mulatta* monkeys, Pub Health Rep *57*, 1126-1139

Ingalls, T H , and Gordon, J E , 1947, The epidemiologic implications of developmental arrests, Am J M Sc *214*, 322-328

Korns, R F , 1952, Prophylaxis of German measles with immune serum globulin, J Infect. Dis *90*, 183-189

Krugman, S , Ward, R , Jacobs, K G , and Lazar, N , 1953, Studies on rubella immunization I Demonstration of rubella without rash, J A M A *151*, 285-288

McLorinan, H , 1950, Diagnosis and prophylaxis of rubella, M J Australia *2*, 390-392

Miller, H , 1956, The neurological complications of the acute specific fevers, Proc Roy Soc Med *49*, 139-148

Mitchell, W , and Pampiglione, G , 1954, Neurological and Mental complications of Rubella, Lancet *2*, 1250-1253

Ober, R. E , Horton, R J M , and Feemster, R. F , 1947, Congenital defects in a year of epidemic rubella, Am J. Pub Health *37*, 1328-1333

Swan, C., 1944, A study of three infants dying from congenital defects following maternal rubella in the early stages of pregnancy, J. Path & Bact *56*, 289-295

———, Rubella in pregnancy as an etiological factor in congenital malformation, stillbirth, miscarriage and abortion, J. Obst & Gynaec Brit Emp *56*, 341-363, 591-605

Swan, C , Tostevin, A L , and Black, G H B , 1946, Final observations on congenital defects in infants following infectious diseases during pregnancy, with special reference to rubella, M J Australia *2*, 889-908

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37

Exanthem Subitum and Erythema Infectiosum

(**Exanthem subitum,** SYNONYMS: Roseola subitum, roseola infantilis, roseola infantum, pseudorubella, exanthem criticum, sixth disease, rose rash of infants, Zahorsky's disease **Erythema infectiosum,** SYNONYMS Duke's disease, fourth disease, fifth disease, parascarlatina, *ringel rötheln, örtliche rötheln*, megalerythema epidermicum, grossflecken, *exantheme variable*, erythema simplex marginatum, erythema infantum febrile, *epidemische kindervotlauf*)

EXANTHEM SUBITUM

INTRODUCTION

Exanthem subitum is a mild but acute illness with sudden onset, occurring for the most part in infants and young children It is mildly contagious, and epidemics have been described particularly in orphan homes or maternity hospitals Characteristically, there is fever and malaise of 3 to 5 days duration, which tends to terminate abruptly on appearance of the exanthem The prognosis is excellent

HISTORY

The disease probably has been known for over a century, but its existence was obscured by the many other mild exanthems with which it was confused (see below). It was characterized as a separate disease entity by Zahorsky (1910) in the United States This report was followed immediately by reports from many parts of the world.

CLINICAL PICTURE

Although most cases occur in the first few months or 2 years of life, cases are not rare up to 4 years, and 1 case has been described in a 31-year-old woman Both sexes are equally affected. The onset is usually sudden with rise in temperature to 105° or 106° F, this abrupt onset may be preceded by a mild inflammation of the nose, the throat and the middle ear and by a cough (McQuitty, 1955) The high fever may induce convulsions. Fever lasts up to 5 days, is of intermittent or remittent type and usually disappears abruptly, together with other symptoms, on appearance of the typical rash Other common symptoms are malaise, irritability and diarrhea Mild meningismus is seen occasionally during the height of the fever. Clemens (1945) has described an enanthem on the soft palate which McQuitty (1955) says occurs during the febrile period The exanthem, which appears only rarely before the disappearance of fever, is typically finely macular but may be maculopapular Where thickest, on thighs and buttocks, it tends to be surrounded by a pale halo It appears first on the trunk, later on neck and arms. Rash is rare and sparse on

the face. The exanthem fades rapidly without desquamation or pigmentation. Cases without rash but with high fever (roseola sine eruptione) occur and cause difficulties in diagnosis. The disease may recur after apparent recovery Leukopenia with absolute lymphocytosis is found particularly in the middle of the febrile period Such lymphocytosis may be accompanied by slight enlargement of the lymph nodes Monocytes may be increased

Etiology

The causative agent is not definitely known Attempts to demonstrate a bacteriologic etiology have not been successful. Kempe et al (1950) have transmitted the disease from one infant to another by intravenous injection of bacteria-free serum collected on the third but not the first day of fever After an incubation period of 9 days, typical exanthem subitum occurred The same authors apparently transmitted the disease to monkeys from both donor and recipient children by means of serum or throat washings taken on the third day of fever. Incubation in the monkey was 4 to 5 days A febrile response was induced in other monkeys by serum taken on the second day of fever and inoculated either untreated or after Seitz (EK) filtration. After the febrile episode the monkeys were refractory for at least 9 days Neva and Enders (1954b) isolated a virus cytopathic for both epithelial and fibroblastic human cells from a case clinically resembling exanthem subitum. Antibodies to the virus appeared in the convalescent serum of the patient Other studies indicate that the agent belongs to the adenovirus group

Diagnosis

Exanthem subitum must be distinguished from measles, rubella, scarlet fever, infectious mononucleosis and dengue among the well-characterized diseases but also from a large number of ill-defined exanthematous conditions (see below). From measles it may be distinguished by mildness, absence of Koplik spots and distribution of rash. The abrupt termination of fever before appearance of rash distinguishes it from all the characterized diseases listed above From rubella it is also distinguished by its abrupt onset. Scarlet fever presents a severe angina, a different hematologic picture and a type of rash entirely different, especially as regards the typical residual staining and marked desquamation. Dengue differs in its secondary rise of temperature and intense pains in bones, joints and muscles Infectious mononucleosis presents an entirely different blood picture and is characterized by the appearance of heterophile antibodies The rash of exanthem subitum must also be distinguished from that produced by sulfonamides and certain other drugs

Treatment

The treatment is entirely symptomatic, and the prognosis is excellent.

Epidemiology

The disease tends to occur in spring and fall. The incubation period is about 10 days with a range of from 7 to 17 The disease can assume epidemic form, and as many as 30 per cent of contacts in the infant age group may show overt disease Nevertheless, it does not usually spread in families, and many general practitioners are apt to regard it as noncontagious. In view of the occurrence of the disease without rash, and the frequent transient nature of the rash when it does occur, it appears to the author that the low contagiousness is actually only apparent and results from a high proportion of subclinical or unrecognized infections.

Control Measures

No special control measures are indicated

ERYTHEMA INFECTIOSUM

Introduction

Besides the exanthematous diseases discussed in this and the preceding two chapters—measles, rubella and exanthem subitum—there are many others of similar clinical nature seen by the pediatrician, which from their characteristics are in all probability of viral origin In practice the numerous attacks of mild exanthemata seen in childhood are labeled atypical and second (or third) attacks of one of these 3 well-recognized diseases, particularly rubella. The evidence for such second attacks is scanty, and indeed their occurrence is inherently improbable It is

clear that we are now in an era when many of the diseases in this indeterminate group will be characterized and their etiology established Already at least one has been shown to be due to an ECHO virus, and Neva and Enders (1954a) have described a cytopathic virus, which may be cultivated in human fibroblasts and was isolated from an epidemic of a mild rubellalike exanthem appearing near Boston in 1951 The cytologic changes were neutralized by convalescent sera of patients. Subsequently, similar epidemics—with in some cases isolation of the same virus—have been seen in Pittsburgh and elsewhere

One disease of this group which presents a characteristic clinical picture, which when looked for is found surprisingly frequently and from which a virus has recently been isolated on more than one occasion, is erythema infectiosum This virus will be discussed briefly here.

The disease is mildly contagious with at most only insignificant malaise or febrile response and indeed frequently with no other symptom or sign besides the characteristic rash

History

The disease was first described in Austria by Escherich in 1886, and shortly thereafter many other epidemics were recognized in Central Europe. Subsequently, the disease has been described in typical form in most countries of the world

Clinical Picture

The disease occurs characteristically in children of from 4 to 12 years of age, but occasional cases are seen in infants and also in adults in whom the symptoms may be slightly more severe Typically, the disease begins with the appearance of rash, since subjective symptoms are very slight and often are absent Rash first appears on the cheeks, which become fiery red and appear windblown They may be burning and even painful The margin of the red area is sharply delimited and raised, resembling erysipelas Within the cheek lesion isolated papules and vesicles may appear and later become crusted There is circumoral pallor Later, rash appears on the limbs and to a lesser degree on the trunk This rash starts as discrete macules which increase rapidly in size and then fuse At the same time the central area fades, leaving a typical lacework pattern of geographic outlines The rash is characteristically evanescent, but even months after its initial disappearance it can be reactivated by heat, friction or other means Itching is an occasional complaint There is no staining or desquamation Complicating herpes simplex is seen occasionally. There may be slight malaise, sore throat, weakness and low-grade fever in an occasional child Since relapse is characteristic, the disease may last several weeks or even months without, however, producing any real inconvenience The blood picture is normal; lymph nodes are not enlarged In exceptional cases a definite prodromal state with fever, coryza, malaise and nausea and diarrhea has been described

Etiology

The clinical characters and the epidemiology of the disease suggest a viral etiology However, until 1955 no etiologic agent had been isolated In the spring of 1957, Werner et al. studied an epidemic in a school in Pennsylvania A cytopathic agent was isolated in 3 instances from stools and throat washings by repeated passage in monkey kidney cells The changes in the growing cells were characteristic with the appearance of giant multinucleated cells containing intranuclear acidophilic inclusions Complement-fixation tests carried out with the tissue culture agent showed an increase in titer of convalescent over acute-phase sera Subsequently, the new viral agent has been grown and produced cytopathic changes in human amniotic cells In 1956, Henle accomplished the isolation in monkey kidney cells of 2 strains of a similar agent from throat swabs of cases of erythema infectiosum then occurring in another town in southeast Pennsylvania (personal communication)

Diagnosis

This exanthem has to be differentiated from the pool of uncharacterized exanthemata discussed above and also from scarlet fever, rubella, measles and exanthem subitum. However, when it occurs in epidemic form such differentiation is easy The exanthem is typi-

the face The exanthem fades rapidly without desquamation or pigmentation. Cases without rash but with high fever (roseola sine eruptione) occur and cause difficulties in diagnosis The disease may recur after apparent recovery Leukopenia with absolute lymphocytosis is found particularly in the middle of the febrile period Such lymphocytosis may be accompanied by slight enlargement of the lymph nodes Monocytes may be increased

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38

The Herpes Virus Group

INTRODUCTION

A group of viruses having similar characteristics has been designated Herpesvirus (Andrewes, 1954) Three of the group members are pathogenic for man *Herpesvirus varicellae* is considered elsewhere (Chap. 39) *Herpesvirus hominis* (Virus of Herpes Simplex) and *Herpesvirus simiae* (B Virus) are considered here

Herpesvirus hominis is a common parasite of man, because of long association, a mutually satisfactory modus vivendi has been established whereby the parasite only infrequently incapacitates its host and thus assures itself of wide distribution This is in contrast with the severe infections it produces when artificially introduced into other hosts, such as the rabbit In the same way *Herpesvirus simiae* infection is usually subclinical in monkeys, but accidental infection of man leads to fatal disease

The physician is concerned with the diagnosis and the care of the small number of human beings in whom *Herpesvirus hominis* produces a protean picture of clinical disease, or in whom an accidental infection with *Herpesvirus simiae* has taken place

The common characteristics of infection by these 2 viruses in their natural hosts are (1) tissues preferentially but not exclusively attacked are those derived from the embryonic ectoderm, (2) superficial vesicles characterize the visible lesions, (3) histologically, the infected cells show intranuclear inclusions; (4) the viruses are readily isolated from infected tissues.

HERPESVIRUS HOMINIS

(SYNONYM: Virus of Herpes Simplex)

HISTORY

Lowenstein, 1919, was the first to publish evidence that a virus from human herpetic keratitis and from the vesicles of herpes labialis would produce specific lesions on the cornea of a rabbit. He credited Gruter with priority for similar unpublished experiments in 1912 and 1913. Gruter, 1920, described the successful transmission of the disease from an experimentally infected rabbit back to the normal cornea of a blind man

In 1920, it was found that herpesvirus could cause encephalitis in rabbits; Doerr, 1920; and in the same year, an active agent with the characteristics of herpesvirus was isolated from a patient who had died of von Economo's encephalitis, Levaditi and Harvier, 1920 This gave rise to the idea that herpes virus might be a less-virulent varient of the "virus of encephalitis," Levaditi, 1922. However, with increased experience it is now known that while *Herpesvirus hominis* is one of many viruses that can cause human encephalitis, it is not the cause of an epidemic variety

Andrewes and Charmichael (1930) observed that a large proportion of normal adults had in their blood neutralizing anti-

cal both in its original form and in its tendency to disappear and reappear frequently over long periods. Moreover, this typical exanthem is associated with almost complete absence of other symptoms or signs in the majority of cases

Treatment

No treatment is necessary or would be of any effect.

Epidemiology

From the earliest described cases it has been recognized that the disease occurs in epidemic form. The incubation period appears to be from 6 to 14 days Morbidity is high in exposed groups, and over 50 per cent of exposed children of 12 years or under may be expected to show symptoms Adult cases occur but with less frequency, presumably because of previous exposure Both sexes are equally affected Infection with this viral exanthem does not protect against rubella or any other recognized viral disease

Control Measures

No special control measures are indicated

REFERENCES

Clemens, H H, 1945, Exanthem subitum (roseola infantum), J Pediat 26, 66-77

Kempe, C H, Shaw, E B, Jackson, J R, and Silver, H K, 1950, Studies on the etiology of exanthem subitum (roseola infantum), J Pediat 37, 561-568

McQuitty, E L, 1955, Roseola infantum, Brit M J 1, 1005-1006

Neva, F A, and Enders, J F, 1954a, Cytopathogenic agents isolated from patients during an unusual epidemic exanthem, J Immunol 72, 307-314

———, 1954b, Isolation of a cytopathogenic agent from an infant with a disease in certain respects resembling roseola infantum, J Immunol 72, 315-321

Werner, G H, Brachman, P S, Ketler, A, Scully, J, and Rake, G, 1957, A new viral agent associated with erythema infectiosum, Ann New York Acad Sc 67, 338-345

Zahorsky, J, 1910, Roseola infantilis, Pediatrics, 22, 60-64

A very alarming complication of eczema infantum . . . is an acute outbreak of numerous vesicles, partly scattered and partly arranged in groups. The vesicles are as large as a lentil, filled with clear serum, and the majority are umbilicated They look like varicella vesicles but undoubtedly do not belong in this class . . The patients have high fever, —40° C or more, and are very restless The vesicles develop very acutely (sometimes overnight), in large numbers and often continue to appear in successive crops, for three or four days, or even a week. Those which appear first undergo dessication, rupture and expose the corium or they become encrusted and fall off The largest number of the varicella-like vesicles are found upon the already excematous skin but smaller groups appear on the previously intact skin of the neighborhood The outcome of the peculiar affection is in the majority of cases favorable

This excellent description of a typical case requires only a few additions Severity of the disease may vary widely but tends to be severe in patients under 1 year In some the skin shows only mild vesiculation with minimal systemic symptoms and clears in a few days (Jackson and Dudgeon, 1951) In severe infections vesicles can continue to appear for as long as 9 days, and great areas of skin may be denuded of epithelium through which the patient loses large quantities of fluid, electrolytes and protein Death may occur from untreated dehydration, secondary bacterial infection or shock due to adrenal necrosis (Brain et al , 1957) It is striking to note the absence of itching during the attack and its return after healing has taken place

Infrequently, involvement of the eczematous skin may be a manifestation of recurrent disease In such patients serious systemic involvement is largely prevented by circulating antibodies, even though the local lesions may be extensive (Boake et al , 1951)

Traumatic Herpes

Primary infection occasionally occurs through traumatic breaks (burns, abrasions) in the normal skin of a susceptible child and results in a characteristic illness About 2 or 3 days after the original trauma, the skin lesion becomes erythematous, and vesicles appear The child develops fever, If the trauma is on a limb, crops of vesicles on an erythematous base appear centripetally, probably through lymphatic channels, during the course of the next week, and the regional lymph node becomes enlarged and tender The picture has to be differentiated from that of herpes zoster with its dermatome distribution The illness may last from 10 days to 3 weeks before all lesions have disappeared Herpetic stomatitis has been reported as occurring during the course of the illness, Following recovery, vesicles or even large bullae (Findlay and MacCallum, 1940) are likely to recur at the site of original injury Primary infection of apparently unbroken skin has been reported (Scott et al , 1952)

Diseases of the Mucous Membranes

Acute Herpetic Gingivostomatitis

(SYNONYMS: Acute infectious gingivostomatitis, aphthous stomatitis, catarrhal stomatitis, ulcerative stomatitis, Vincent's stomatitis)

This is the commonest manifestation of primary infection While its peak incidence is between 1 and 3 years, it can occur in older children and adults but it seldom if ever occurs below the age of 12 months During the ages of greatest incidence it is probably the most frequent cause of clinical stomatitis The chief characteristics, which are essentially the same for all ages, are as follows fever, irritability, red swollen gums, a vesicular eruption on the mucous membranes of the mouth, oral fetor and local lymphadenopathy (Scott et al , 1941) In about a third of the cases, the onset is insidious, the child being febrile for 2 or 3 days before a sore mouth develops, in the remainder, there is early refusal to eat or actual complaint of sore mouth A constitutional reaction is always present but varies in severity, a high fever, from 104 to 105° F, is not uncommon Visceral involvement has been demonstrated by liver biopsy in a severe case (Tolentino and Matteis, 1953). Lesions in the mouth vary in size and number and are essentially vesicular, although typical vesicles are rapidly modified by maceration and appear as shiny grayish-yellow plaques representing collapsed vesicles with edematous roof cells These often ulcerate before healing No portion of the oral lining is immune, although the tongue and the cheeks are most commonly involved In

bodies against herpes virus, and that recurrent herpes only occurred in those with neutralizing antibodies Since these findings appeared to be contrary to the usual pattern of infectious disease, and since herpes did not seem to spread from patient to patient but seemed to be provoked by some nonspecific stimulus, such as fever, Doerr (1938) suggested "that herpes is not an infectious agent which is maintained by a chain of infection but that it is endogenously generated in the human organism."

These difficulties were resolved when it was shown by Dodd et al (1938) that herpes virus could be routinely isolated from the mouths of infants suffering from a common form of stomatitis, and by Burnet and Williams (1939) that such infants also developed neutralizing antibodies during convalescence from the stomatitis

Following this, similar proofs of a primary herpetic infection were presented by Seidenberg (1941) and Ruchmann et al (1947) for eczema herpeticum; Slavin and Gavette (1946) for vulvovaginitis, Gallardo (1943) for keratoconjunctivitis and Smith et al. (1941) and Armstrong (1943) for meningoencephalitis. As a result it is now recognized that the herpes virus acts like any other infectious agent in the primary infection of a susceptible host On the host's recovery it appears to become latent in the tissues where it can cause local damage under circumstances altering the host's physiology, despite the presence of antibodies in the circulation.

Clinical Picture

The characteristics of the virus result in 2 types of clinical herpetic disease: (1) primary infections with the virus, occurring in persons without neutralizing antibodies in their blood stream; (2) recurrent disease, occurring in persons with such neutralizing antibodies In a primary infection, the local lesion is usually accompanied by a systemic illness that is often severe and sometimes fatal, whereas the patient with a recurrent attack is as a rule singularly free from systemic symptoms, however severe the local lesion may be Serologic surveys have shown that many more adults have circulating antibodies than can be accounted for by a history of clinical infections This means that the majority of infections are subclinical, only an estimated 10 per cent of primary infections causing clinical illness (Scott, 1957). Recovered individuals from a manifest or a subclinical infection may become carriers of the virus Following nonspecific stimuli, such as fever, menstruation, or emotional upsets, these are apt to have recurrent manifestations of herpetic infection.

The clinical manifestations of herpetic infection may be considered under various disease categories: (1) of the skin, (2) of the mucous membranes, (3) of the eye, (4) of the central nervous system, (5) disseminated infections. Most of these present a clinical picture which can usually be recognized. The virus can be isolated readily from typical visible lesions and sometimes from the saliva of an asymptomatic carrier (Buddingh, et al, 1953). Reported isolation of virus from patients with other clinical pictures must be reviewed in the light of a possible superinfection of damaged tissue by this ubiquitous virus.

Diseases of the Skin

Herpes Simplex (Synonyms: Herpes labialis, facialis, febrilis)

This commonest manifestation of recurrent disease, often involving a mucotaneous junction presents as follows: There is a sensation of irritation or burning at the site involved lasting a few hours Reddish papules then appear which quickly vesiculate When fully developed the lesion consists of one or more areas of grouped thin-walled vesicles on an erythematous base The vesicles quickly become purulent, scab and heal without a scar, unless traumatized Recurrences tend to affect the same area of skin time after time In contrast, when the skin is affected in a primary infection, as, for instance, of the chin of an infant with herpetic stomatitis, the vesicles are widely scattered and single Occasionally, lesions recur in a zosterlike distribution (Slavin and Ferguson, 1950), sometimes with an associated lymphangitis (Trice and Shafer, 1953).

Eczema Herpeticum (Lynch, 1945) (Synonyms: Kaposi's varicelliform eruption, Juliusberg's pustulosis vacciniformis acuta)

This is usually a manifestation of a primary infection in which the skin of the eczematous patient is the portal of entry. The clinical description of this disease by Kaposi in 1887 remains good today (Kaposi, 1895):

small dendritic ulcers, or a vesicle appears, often near the lower border of the cornea, which enlarges in a series of branching processes to form the typical "dendritic" ulcer characteristic of herpetic infection. If untreated, this will continue to increase in size to involve large areas of the epithelium, resulting in a "geographic" ulcer with uneven branching edges Although corneal ulceration may occur in either primary or recurrent disease, the diagnostic differentiation may be suspected clinically, since follicular conjunctivitis, an invariable accompaniment of the primary infection, is only rarely present in the recurrent disease Occasionally, indolent, deep, nonbranching marginal ulcers occur in the recurrent disease These leave a clear rim of uninvolved peripheral cells which are protected by the humoral antibodies in the limbic vessels Large erosions of the cornea may recur in healed areas due to weak adhesion between epithelium and Bowman's capsule In the presence of corneal involvement the acute phase usually subsides in 2 to 3 weeks, although some keratitis may persist for several weeks longer

Deep Forms (Thygeson and Kimura, 1957) In contrast with herpetic infections elsewhere, lesions of the cornea, perhaps because of its avascular structure, almost always involve the stroma, tend to be chronic and are accompanied by scarring The clinical pictures are as follows

Disciform Keratitis This may be benign with edema but not necrosis of the stromal fibers, run a definite course of 2 to 3 months and heal with minimal scarring It may follow a clearly demonstrable or an insignificant superficial lesion More commonly it is severe, with necrosis and sometimes rupture of the cornea followed by healing, with dense scar formation, only after many months or even a year Secondary bacterial infection is an almost invariable accompaniment Absence of pain is characteristic because of the hypalgesia of the cornea in herpetic infections Sometimes the disciform process may extend to involve the whole corneal stroma (herpetic interstitial keratitis)

Hypopyon Keratitis This may follow large "geographic" ulceration, particularly after the use of steroids It must be differentiated from that following deep bacterial and fungal ulcerations, by noting the corneal anesthesia in herpetic in contrast with the pain associated with bacterial or fungal ulcers

Iridocyclitis. This may complicate both disciform and hypopyon keratitis. It is characterized by severe pain, circumcorneal flush and multiple keratic precipitates. Synechia formation and secondary lens opacification are frequent Recurrences may occur following fever or other trigger mechanism without accompanying keratitis

The management of these patients should be directed by an ophthalmologist Of great importance is the observation that steroids used either topically or generally, cause increase in severity and frequency of the deep forms of the disease The only specific therapy recommended for restoration of sight to patients with scarred corneae, subject to recurrent disease, is removal of the diseased tissue and replacement by a corneal transplant

Diseases of the Central Nervous System

Herpetic Meningo-encephalitis Herpetic infection of the central nervous system may cause the clinical picture of aseptic meningitis or of acute encephalitis with coma, ocular palsies, paresis of muscle groups, sensory changes and convulsions When evidence of increased intracranial pressure is found the mortality is high death occurring between the 8th and the 12th days (Whitman et al 1946) About 5 to 7 per cent of meningoencephalides are caused by the virus (Afzelius-Alm, 1951, Adair et al, 1953) The mechanism of spread to the nervous system is not clear Hunt and Comer (1955) report a case in which there is suggestive evidence of propagation along nerve axons An etiologic diagnosis can be suspected on clinical grounds if a characteristic stomatitis or lesions on the skin are present A definitive diagnosis can be made only by laboratory means

Associated with Trigeminal Neuralgia. Recurrent attacks may occur at the time of, or a few days after, an attack of trigeminal neuralgia in the area of distribution of the 2nd and the 3rd divisions of the nerve They are frequent after operative section of

some patients the tonsillar region is affected early. Under these circumstances the lesions must be distinguished from those of herpangina due to Coxsackie virus, nonbacterial exudative pharyngitis due to several adenoviruses and bacterial tonsillitis The unilateral tonsillar ulcers of Vincent's angina, which is not herpetic (Steigman and Scott, 1947), can easily be differentiated. Involvement of the gums is characteristic but varies in intensity; sometimes there is only a thin red line along the dental margin which may be the initial evidence of an herpetic stomatitis in a febrile child; sometimes the gums are extensively swollen In the rare infant, infected before the eruption of teeth, the gums escape involvement Submaxillary lymphadenopathy of varying degree is extremely common The disease is self-limiting in about 1 to 2 weeks Pain tends to disappear before the lesions heal. Scarring is absent or very slight Swelling of the gums and lymphadenopathy, among the first signs to appear, usually persist for some days after the ulcers heal

Recurrent Stomatitis

The oral, in contrast with the genital, mucous membranes do not consistently manifest recurrent lesions, although sometimes these can be found on the inner lip, adjoining gum or, *rarely*, more wide-spread during a recurrent attack of herpes labialis (Farmer, 1956) Nevertheless, a history of recurrent stomatitis should weigh against a diagnosis of herpes Recurrent aphthae are as a rule not due to herpes (Blank et al, 1950, Dodd and Ruchman, 1950). Recurrent nonspecific ulceration as a manifestation of *erythema multiforme* may follow 7 to 10 days after a recurrent attack of herpes labialis

Acute Herpetic Rhinitis. The nose may be the site of primary infection, manifested, in the nostrils, by tiny vesicles surrounded by red areolae, enlarged anterior cervical lymph nodes, and fever (Ruchman and Dodd, 1950)

Herpetic Involvement of the Genitalia

FEMALE. These may show primary or recurrent lesions In a primary infection vesicles appear on the mucous membranes of the labia (both majora and minora) and the lower vagina These are quickly modified, as in the mouth, resulting in scattered lesions covered with gray-yellow membranes Accompanying these are local pain, enlarged regional nodes, low-grade fever and sometimes vesicular lesions on the adjacent skin The lesions heal in the course of 1 to 2 weeks. The recurrent disease shows similar lesions (Slavin and Gavette, 1946).

MALE. Primary infection is apparently very uncommon. It has taken the form of a nonbacterial urethritis, with burning pain on micturition and either a watery or a purulent discharge. Tiny vesicles have appeared on the glans or the prepuce. No regional lymphadenopathy has been reported. In one case a generalized cutaneous hyperesthesia, mainly of the lower limbs, accompanied the peak of the illness, which lasted about 2 months A concurrent herpetic stomatitis was present in one case (Slavin, 1951; Esteves and Pinto, 1952) Recurrent attacks (*Herpes progenitalis*) are quite common and consist of a cluster of erosions (eroded vesicles) on the glans, or the corona, sometimes associated with herpetic vesicles on the shaft of the penis

Diseases of the Eye

Herpetic Keratoconjunctivitis and Keratitis

Herpetic infection of the eye is a serious hazard to sight Clinically, the infection can be confined to the superficial or can involve the deep structures of the eye As in other sites, the clinical manifestations may be due to primary or recurrent disease

SUPERFICIAL FORMS (Braley, 1957, Ormsby, 1957). These are characteristic of acute herpetic keratoconjunctivitis, which is usually a manifestation of primary herpetic infection occurring chiefly in children and adolescents The disease usually begins as a unilateral conjunctivitis with accompanying preauricular lymphadenopathy and malaise It may be associated with a vesicular eruption on the lids or a stomatitis There is a follicular conjunctivitis, with some chemosis and edema of the lids Ulcers of the conjunctiva sometimes are seen The infection may be confined to the conjunctiva, in which case the disease clears up in a few days The cornea may be involved in two ways. There is either an early diffuse epithelialitis, resulting in superficial punctate erosions which later develop into

lesions inclusion bodies are found In the lungs, in addition to this picture, Pugh et al (1955) have described a peculiar type of necrosis, basophilic in character and consisting mainly of deoxyribonucleic acid, which they ascribe to intense local necrosis with rapid breakdown of cells The area is sharply demarcated from the adjacent tissues which show intense congestion and infiltration with mononuclear cells

The pathologic changes in the nervous system occur mostly in the cortex with progressively less involvement of the central white matter and the basilar structures Grossly, there are focal areas of dusky discoloration, studded with petechiae, which are swollen and friable Histologically, there is widespread mononuclear infiltration of the leptomeninges The lesions in the cortex are characterized by intense degeneration and mild inflammation The appearance suggests encephalomalacia due to circulatory disturbance Fat-laden phagocytes flood the field, and the capillaries show endothelial hyperplasia Neuronophagia occurs At the margins of necrotic areas, hypertrophied microglial cells and mild astrocytosis are seen There is perivascular cuffing with mononuclear cells Inclusion bodies may be demonstrated, preferably with phloxine-methylene blue stain, in oligodendria and less frequently in nerve cells (Wolf, 1950)

Experimental Infection, Host Range

The natural host of the virus is man However, it can be transmitted to the following experimental hosts rabbit, guinea pig, mouse, hamster, cotton rat, the embryonated hen's egg and to several types of cells grown in tissue culture of which the most utilized are rabbit kidney, and HeLa and human amnion

In the animal hosts the reaction depends on the route of inoculation of virus When introduced by corneal scarification a keratoconjunctivitis develops in the rabbit and the guinea pig between 12 hours and 7 days, depending on the virulence of the preparation The conjunctiva becomes injected, the cornea cloudy, because of the development of small vesicles along the scratch marks, and an exudate appears, at first watery but later purulent, in which many of the cells are mononuclear, although not predominantly so, and there are no bacteria The nictitating membrane becomes red and swollen The lesion heals slowly over the course of 1 to 2 weeks, leaving a varying amount of residual corneal opacity.

The encephalitis resulting from intracerebral inoculation manifests itself by fever, tremors, lethargy, convulsions and weakness of muscle groups Death usually ensues, although recovery may take place An entirely comparable encephalitis occurs in the rabbit after the corneal inoculation of certain neurotropic strains Suckling mice 1 to 3 days old are particularly susceptible and die on 5th to the 6th day after intracerebral and intraperitoneal inoculation with weakness, in-coordination and paralysis (Kilbourne and Horsfall, 1951, Lennette and van Allen, 1957) Certain strains produce characteristic lesions in the skin of the rabbit or the foot pads of the guinea pig About 2 days after an intradermal inoculation a red papular lesion develops which may vesiculate and then subsides after 2 to 3 days

The embryonated hen's egg is a very susceptible host Inoculation onto the chorioallantoic membrane of 11- to 13-day-old eggs gives rise to characteristic pocks which appear as early as the 20th hour but are best examined after incubation at 36° C from the 36th to the 48th hours The pocks are small, 1 to 2 mm in diameter, white, often oval and sometimes with tails, raised above the ectoderm Central necrosis and mesodermal reaction may be seen in old lesions, but these are not very conspicuous in contrast with the lesions of vaccinia (Beveridge and Burnet, 1946) Histologic examination of an early pock, 20 hours after inoculation, reveals that it consists of a heap of proliferated cells, 90 per cent of which contain Fuelgen positive inclusions filling the nuclei During the ensuing 72 hours the following changes occur the epithelial cells slough, remaining inclusions become shrunken, the mesoderm shows an inflammatory reaction and some inclusion bodies and, finally, the entoderm becomes thickened by cell proliferation (Crouse et al, 1950)

The blastoderm of 1-day-old eggs is equally susceptible, after infection the blastoderm ceases to develop, but virus continues to be formed over the course of 5 days (Yoshino, 1956).

Inoculation of virus into the yolk sac of 9-day-old eggs causes death of the embryo

the nerve It is not certain whether the virus lies latent in the nerve tissue or the skin, but more likely the latter (*Carton and Kilbourne*, 1952; Behrman and Knight, 1954).

Disseminated Herpes

Viremia has been demonstrated occasionally in severe cases of primary herpetic infection (Ruchmann and Dodd, 1950), and evidence of infection of the liver has been found in children with stomatitis (Tolentino and Matteis, 1953, Zuelzer and Stulberg, 1952) Therefore, it is not surprising that occasionally widespread clinical manifestations of the infection appear.

Neonatal. There have been several reports of a fatal infection in newborns (Quilligan and Wilson, 1951; Zuelzer and Stulberg, 1952, Pugh et al., 1954; Williams and Jack, 1955). The illness may start between the 4th and the 7th days after birth, with vesicles on *the skin, a stomatitis or a conjunctivitis.* Following this, the infant becomes seriously ill with high fever, dyspnea, evidence of encephalitis, and jaundice and dies within a few days. Death with generalized symptoms of pulmonary, liver or central nervous system disease may occur without any evidence of superficial herpetic infection At autopsy hepatic necrosis is a striking and sometimes the only finding; cells in the vicinity of the necrotic areas contain intranuclear inclusions In other patients similar intranuclear inclusions have been found in the endothelium of the blood vessels, lungs, spleen, kidneys, adrenals and brain. Herpes virus has been isolated from the liver of several such patients Death may occur from a secondary septicemia of *Ps. pyocyanea* The pathogenesis of these infections is obscure The infant may be exposed to virus from the mother, suffering a primary infection around the time of delivery, or, if born of a mother without antibodies, to virus from an attendant, either a carrier or with a recurrent lesion. Prematurity seems to play a role, the exact nature of which is not known.

Adults. Kilbourne and Horsfall (1951) described 2 adults with symptoms suggestive

initially diagnosed as suffering from smallpox because of severe systemic illness and a widespread rash (Kipping and Downie, 1948).

Pathologic Picture

Details of the pathologic picture vary with the type of infected tissue In general, the characteristic change induced by the virus is the intranuclear inclusion body first described by Lipshutz, 1921. The development of the inclusion body appears to be a dynamic occurrence. One or more areas of Feulgen-positive material appear between strands of condensed chromatin, these coalesce to form a Feulgen-positive homogenous body. This appears bluish with routine hematoxylin and eosin stains and fills the nucleus, compressing the c

shrir

nega

by a halo from the marginated chromatin (Crouse et al., 1950, Wolman and Behar, 1952) This is now the classic Type A inclusion body of Cowdry, 1934.

Pathologic changes in the skin and the mucous membranes are characterized by vesicle formation In the latter the vesicle is modified by early maceration and leakage of fluid These changes take place in the

characteristic multinucleated giant cells, leukocytes and fibrin Each giant-cell nucleus contains an inclusion body These giant cells are of diagnostic aid and can be recognized in a smear from the base of a fresh vesicle. The infected epithelium at the edge of the vesicle can be distinguished from the normal skin by the presence of proliferation, "ballooning degeneration" and intranuclear inclusions The mucosal lesion resembles the skin lesion except for the relative absence of fluid, greater amount of fibrin and edematous thickening of the roof-cells in the former. The corium of both skin and mucous membrane shows pronounced capillary dilatation and infiltration of inflammatory cells but no necrosis, which accounts for the usual absence of scarring.

In disseminated infection many viscera are involved, the liver and the adrenals show *coagulation necrosis involving not only parenchymal* cells but also stroma and blood vessels with little cellular inflammatory response. In the cells at the periphery of these

medium through breaks in the cell membrane These double membrane particles, representing mature virus, measure 120 to 130 mμ in diameter (Morgan et al, 1954) It would appear from the above and from other circumstantial evidence that *Herpesvirus hominis* is released from the infected cell by a slow leak rather than by disruption of the cell

Latency. This is a characteristic of the virus in vivo Pelmont and Morgan (1958) have reproduced this in vitro, in that when infected HeLa cells are carried in a deficient medium the virus can no longer be detected but becomes demonstrable again when the nutritional deficiences are corrected

Antibody Response Complement-fixing neutralizing and skin-test antibodies are formed by the intact host. After primary infection these first two appear between 4th and the 6th days, reaching their height by the 14th day In children the antibody titer may drop to undeterminable levels after the first infection (Buddingh et al, 1953), only to be boosted by a series of subclinical or possibly clinical reinfections

Preservation The activity of the virus is temperature dependent in that a strain may have a ½ life of 5 hours at 37° C and 24 hours at 30° C Inactivation at 37° C is increased when the pH of the medium is outside the range of 6 8 to 7 4, especially in the lower ranges (Stoker, 1958) Activity is also destroyed by lipid solvents such as ether and fluorocarbon, and by phosphatases It can be preserved in whole tissue in 50 per cent glycerol, or at −70° C in the presence of stabilizers such as skim milk, serum or egg yolk (Scott, 1956)

By adulthood the CF and neutralizing antibody levels in a given individual have usually been stabilized As a result they do not vary with the clinical appearance of recurrent disease, although rises may occur in an occasional individual Skin test antibodies appear to parallel the other two between the ages of 10 and 50 years but are inconsistent at the extremes of life (Scott, 1957)

Diagnosis

This is based on the combination of a characteristic clinical picture and a positive laboratory diagnosis The latter consists of (1) the isolation of the virus from a lesion or a tissue of the patient as a result of inoculation into a susceptible host, (2) the demonstration of a rise in antibodies during convalescence from a primary infection (the neutralization antibody is the most constant); (3) the demonstration of virus-giant cells in scrapings from the base of a fresh lesion (Blank et al, 1951), or (4) demonstration of inclusions in tissue sections

Infections by this virus must be differentiated from those by several other viruses While the laboratory must provide the final answer, there are certain helpful clinical criteria as follows.

1 *Eczema Herpeticum from Eczema Vaccinatum* In the former, vesicles appear in crops so that the rash shows lesions of different stages at any given time while in the latter they tend to be all at the same stage In the latter there is almost always a history of exposure to vaccinia while in the former there may be a cold sore in one of the contacts The same criteria can be applied to the differentiation between *herpetic and vaccinal vulvitis*

2 *Herpetic Keratoconjunctivitis from Epidemic Keratoconjunctivitis (Adenovirus Type 8)* The latter is painful whereas relative anesthesia of the cornea is characteristic of the former A history of recurrences, the presence of typical dendritic ulcer and vesicles on the lids are also characteristic of the former

3 *Acute Herpetic Gingivostomatitis from Herpangina (Coxsackie Group A Viruses) and Nonbacterial Exudative Pharyngitis (Adenoviruses)* This may be particularly difficult in those cases in which the herpetic infection begins in the fauces, which is the characteristic place for the lesions of Herpangina However, in the latter the lesions do not spread to other portions of the buccal mucosa which are eventually involved in the herpetic infection and the course of Herpangina is much shorter (4 to 6 days) than that of herpetic stomatitis Herpangina is a summer disease, and there is a frequent history of contact, while herpetic infection occurs at all seasons, and usually there is no history of contact (Parrott et al, 1954). Suspicion of adenovirus infection may be supported if there are present conjunctivitis or generalized lymphadenopathy

after an incubation period of about 48 to 72 hours

Infected rabbit kidney cells in culture undergo the following changes: Cytoplasmic granulation appears, and the cells become rounded and refractile, "balloon" cells, multinucleate giant cells appear, in which the nuclei may be pyknotic or contain inclusion bodies; finally, areas of lysis occur (Sosa Martinez et al, 1955) Rather similar changes are described for HeLa cells (Scherer and Syverton, 1954) Human amnion cells show much the same response. The giant cells arise largely from cell fusion and may contain hundreds of nuclei, each containing an inclusion body (Stoker, 1958).

The strain of virus used may determine whether clumping and apparent pock formation, or giant cells and lysis occur predominantly (Gray et al, 1958) Rabbit kidney tissue culture sheets can be used for quantitating the virus, since infected areas appear as white plaques when the culture is stained with neutral red (Kaplan, 1957)

Etiology

Appearance and Size. The electron-microscopic appearance is that of an inhomogenous sphere with a dense center However, in some particles derived from egg or vesicle fluid the center appears less dense and almost absent, resulting in doughnut-shaped forms (Coriell et al, 1950) Filtration through gradocol membranes revealed an average diameter of 100 to 150 mμ (Elford et al, 1933) while Munk and Akerman (1953) suggested a diameter of 96 mμ based on calculations [illegible]

and Hunt, 1954) and different genetic markers (Wildy, 1955)

Course of Infection. In HeLa cells the following stages have been described (Stoker, 1958). Adsorption occurred slowly, about 50 per cent being adsorbed in 1 hour and 80 per cent by 2 hours By the end of 2 hours about half the absorbed virus had entered the cell and inhibited mitosis; an eclipse phase then ensued, the length of which depended on the amount of virus entering the cell but was not less than [redacted] hours. With the electron microscope viral particles in large numbers could be seen attached to the cell surface during the eclipse phase which could be removed by specific antisera or EDTA, new virus particles appeared in the nucleus at 12 hours, and large numbers were present in the cytoplasm and on the cell surface by 26 hours Free virus appeared in the medium by 15 hours; this corresponded to the formation of about 100 particles per cell by 50 hours In addition, it seemed clear that some form of virus could spread from cell to cell without entering the medium.

DNA increased 40 per cent in the nuclei of infected cells during the eclipse phase when no recognizable particles were seen under the electron microscope and was almost double the normal quantity by 72 hours The source of this DNA is not clear, since it is more than could possibly be of viral origin even if herpes virus were a DNA virus, which is not yet known (Newton and Stoker, 1958).

These findings are in general agreement with the less detailed observations reported after infection of the C A M where the rate of adsorption was somewhat more rapid, 50 to 80 per cent being adsorbed in 15 minutes, and the eclipse phase lasted 7 to 12 hours This was followed by a logarithmic release of infectious virus into the medium over the next 10 to 12 hours (Scott et al, 1953b)

In the C.A M it would appear that 1 infectious virus particle was equivalent to 1 pock-forming unit (Yoshino and Taniguchi, 1956)

Relation of Virus to Intranuclear Inclusion Body. The nature of the inclusion body now appears to be established as an accumulation of developing virus particles The eclipse period corresponds to progressive changes in the nucleus up to the development of an inclusion that fills the nucleus At this time infectious virus is again detectable Thereafter, virus gradually leaves the nucleus, and the classic Type A inclusion represents a virus-free residue (Scott et al, 1953a, Lebrun, 1956). Under the electron-microscope, dense particles measuring 30 to 40 mμ, appear in the infected nucleus These enlarge and acquire a single membrane reaching the size of 70 to 100 mμ. Larger particles with 2 surrounding membranes then seem to make their way into the cytoplasm through breaks in the nuclear membrane and into the

medium through breaks in the cell membrane These double membrane particles, representing mature virus, measure 120 to 130 mμ in diameter (Morgan et al, 1954) It would appear from the above and from other circumstantial evidence that *Herpesvirus hominis* is released from the infected cell by a slow leak rather than by disruption of the cell

Latency. This is a characteristic of the virus in vivo Pelmont and Morgan (1958) have reproduced this in vitro, in that when infected HeLa cells are carried in a deficient medium the virus can no longer be detected but becomes demonstrable again when the nutritional deficiences are corrected

Antibody Response. Complement-fixing, neutralizing and skin-test antibodies are formed by the intact host After primary infection these first two appear between 4th and the 6th days, reaching their height by the 14th day In children the antibody titer may drop to undeterminable levels after the first infection (Buddingh et al, 1953), only to be boosted by a series of subclinical or possibly clinical reinfections

Preservation The activity of the virus is temperature dependent in that a strain may have a ½ life of 5 hours at 37° C and 24 hours at 30° C Inactivation at 37° C is increased when the pH of the medium is outside the range of 6 8 to 7 4, especially in the lower ranges (Stoker, 1958) Activity is also destroyed by lipid solvents such as ether and fluorocarbon, and by phosphatases It can be preserved in whole tissue in 50 per cent glycerol, or at −70° C in the presence of stabilizers such as skim milk, serum or egg yolk (Scott, 1956)

By adulthood the CF and neutralizing antibody levels in a given individual have usually been stabilized As a result they do not vary with the clinical appearance of recurrent disease, although rises may occur in an occasional individual Skin test antibodies appear to parallel the other two between the ages of 10 and 50 years but are inconsistent at the extremes of life (Scott, 1957)

Diagnosis

This is based on the combination of a characteristic clinical picture and a positive laboratory diagnosis The latter consists of (1) the isolation of the virus from a lesion or a tissue of the patient as a result of inoculation into a susceptible host; (2) the demonstration of a rise in antibodies during convalescence from a primary infection (the neutralization antibody is the most constant); (3) the demonstration of virus-giant cells in scrapings from the base of a fresh lesion (Blank et al, 1951), or (4) demonstration of inclusions in tissue sections

Infections by this virus must be differentiated from those by several other viruses While the laboratory must provide the final answer, there are certain helpful clinical criteria as follows

1 *Eczema Herpeticum from Eczema Vaccinatum* In the former, vesicles appear in crops so that the rash shows lesions of different stages at any given time while in the latter they tend to be all at the same stage In the latter there is almost always a history of exposure to vaccinia while in the former there may be a cold sore in one of the contacts The same criteria can be applied to the differentiation between *herpetic and vaccinal vulvites*

2 *Herpetic Keratoconjunctivitis from Epidemic Keratoconjunctivitis (Adenovirus Type 8)* The latter is painful, whereas relative anesthesia of the cornea is characteristic of the former A history of recurrences, the presence of typical dendritic ulcer and vesicles on the lids are also characteristic of the former

3 *Acute Herpetic Gingivostomatitis from Herpangina (Coxsackie Group A Viruses) and Nonbacterial Exudative Pharyngitis (Adenoviruses)* This may be particularly difficult in those cases in which the herpetic infection begins in the fauces, which is the characteristic place for the lesions of Herpangina However, in the latter the lesions do not spread to other portions of the buccal mucosa which are eventually involved in the herpetic infection and the course of Herpangina is much shorter (4 to 6 days) than that of herpetic stomatitis Herpangina is a summer disease, and there is a frequent history of contact, while herpetic infection occurs at all seasons, and usually there is no history of contact (Parrott et al, 1954) Suspicion of adenovirus infection may be supported if there are present conjunctivitis or generalized lymphadenopathy

4. Herpetic Meningo-encephalitis from the Numerous Other Viral Encephalitides This is almost impossible in most instances The epidemiologic history may be some guidance in ruling out certain other viruses such as the arthropod-borne group, or the patient may show other signs of herpetic infection

Treatment

There is no specific treatment, and the clinical manifestations of the disease are self-limited However, nonspecific supportive therapy must be utilized in primary infection to preserve life in some instances and in recurrent infection to give symptomatic relief and, in eye infections, to preserve sight

Primary Infections. Severe stomatitis may lead to dehydration in infants because of lack of intake This must be combated by appropriate parenteral fluid and electrolyte replacement Intake of fluid and food must be encouraged, cold semisolids like ice cream are often better tolerated than plain liquids at room temperature Local anesthetics before feeding may be helpful in older children Prevention of secondary bacterial infection may be aided by the frequent use of mouth washes such as Ceepryn 1 : 4,000 or a freshly prepared suspension of chlortetracycline, 1 to 3 per cent, in 10 per cent glycerine containing 100,000 units of Nystatin per ml. This can be used to swab the mouths of infants. Systemic penicillin in prophylactic doses may be useful in the younger children where local therapy is difficult

Severe eczema herpeticum requires meticulous attention Parenteral replacement of the fluid, electrolytes and protein lost through the denuded skin is required Anticipation of a secondary bacterial infection requires appropriate and sustained antibiotic therapy based on culture results Local care of the skin requires removal of crusts by soaks, and application of nonsensitizing antibiotics, e g , chloramphenicol, in a hydrophilic ointment base. When there is a severe systemic reaction, steroids *in full doses for a few days may tide* the patient over a crisis Gamma globulin has [illegible] *of the disease* by providing antibodies.

Encephalitis. A patient with herpetic encephalitis must be managed according to the principles utilized in the case of any acute encephalitis

Recurrent Infection. The occasional recurrent vesicle can be treated with local measures. Drying agents, such as alcohol, or Ceepryn 1:4,000, are useful for early application to lesions of the lips or the skin, followed by a broad-spectrum antibiotic ointment (e g , Neosporin). Permanganate sitz baths provide cleanliness and comfort for vulval lesions. The patient that is plagued with very frequent recurrences, unassociated with a clearly recognizable trigger mechanism may require psychotherapy (Blank and Brody, 1950). The application of superficial x-rays is not justified The use of repeated courses of smallpox vaccination has a widespread current vogue but seems to have little theoretical basis Beneficial results are reported by some, but not all, observers The mechanism is unknown and may possibly be based on suggestion Vaccination with herpes virus, either dead (Anderson et al , 1950) or living, is theoretically unsound and practically useless It may be hazardous if live virus is used (Lazar, 1956).

The treatment of eye lesions should be under the supervision of an ophthalmologist. This consists of cauterization and scraping of the corneal ulcer, prevention of secondary bacterial infection by means of topical antibiotic or chemotherapeutic applications and keratoplasty in hopelessly damaged corneae.

Epidemiology

Two separate population groups have to be considered (1) those without circulating antibodies who are susceptible to primary infection, and (2) those with circulating antibodies who are liable to recurrent disease (Scott, 1957).

Primary infection. Pertinent factors may be classified under 3 heads: (1) *Socio-economic and environmental.* It was early noted that the incidence of infection was considerably higher among persons in a lower than those in a higher socio-economic bracket. The amount of overcrowding and the level of public health education appear to increase and decrease, respectively, the incidence in a given population. (2) *Age.* The number of

immunes in a population, as measured by the presence of detectable humoral antibodies, varies with age. The percentage of immune newborns is the same as that of the adults but decreases to a low level between the ages of 6 months and 2 years, thereafter increasing to that of the adult population by about the age of 5 years. The incidence of clinical disease is greatest between 1 and 3 years. For unexplained reasons herpetic stomatitis is almost unknown under 1 year, although eczematous infants of less than 1 year not infrequently suffer an attack of eczema herpeticum. (3) *Reservoir of Infection*. This must be largely among subclinically infected carriers, since actual contact with a clinical infection is relatively rare, being variously estimated from 10 to 50 per cent. It has been demonstrated that patients completely recovered from stomatitis may excrete virus in their saliva intermittently for as long as 7 weeks and that virus can be isolated from the saliva of 2.5 per cent of asymptomatic adults. It can also be isolated from the stools.

Incubation period. Evidence from various sources suggests a range between 2 and 12 days with a peak at 4 days.

Epidemics. As noted earlier, this is an endemic disease with about 90 per cent of the infections being subclinical. However, under exceptional circumstances epidemics can occur, (1) in families in which more than one member may be affected either at the same time from a common source or in sequence, (2) in a hospital ward where patients with eczema can be infected following the admission of a case of eczema herpeticum, (3) in institutions as, for example, one in which 20 of 36 infants under 22 months developed herpetic stomatitis and 16 had subclinical infection (Anderson and Hamilton, 1949).

Mechanism of Spread. This appears to be by contagion in which spread is favored by overcrowding, and the close bodily contact that occurs in infancy, and among adults in kissing and sexual intercourse. Trauma appears to be an important trigger mechanism in establishing a clinical infection in a susceptible individual.

Recurrent Disease. The basic stimulus for the appearance of a recurrent lesion is not a reinfection from without but a disturbance in the physiology of the host which activates a latent virus. This may be induced by a change in (1) the external environment, such as exposure to ultraviolet rays or (2) the internal environment, such as produced by fever, menstruation, nerve injury, or emotion.

Control Measures

There are no specific control measures apart from generally accepted standards of hygiene. It is particularly important for persons suffering from atopic eczema to avoid exposure, and patients with eczema herpeticum must be rigidly isolated. For the susceptible adult, sexual contact in some form probably provides the greatest risk of primary infection.

HERPESVIRUS SIMIAE
(Synonym: B virus)

History

In 1934 Sabin and Wright reported the isolation of this virus from the brain of a laboratory worker dying 18 days after the bite of an apparently normal *Macacus rhesus* monkey. It was found to be immunologically related to the viruses of herpes simplex and pseudorabies (Sabin, 1934). A second fatal case was diagnosed in 1949 (Sabin, 1949). Since that time a number of laboratory workers have been infected, the virus being isolated from their central nervous system, or their blood showed a significant rise of specific antibodies. It has also been isolated from the nervous system of "normal" monkeys and from monkey kidney cells in tissue culture (Wood and Shimada, 1954, Melnick and Banker, 1954).

Clinical Picture

The clinical features of the patients observed have shown the same variability expected in any central nervous system infection. Thus there have been symptoms and signs of (1) acute encephalitis with headache, vomiting, early coma and early death with evidence of increased intracranial pressure but little else abnormal on neurologic examination, (2) encephalomyelitis with excitement, confusion or delirium, and sometimes hypertension, cranial and spinal nerve involvement resulting in diplopia, nystagmus, patchy paresthesia of head, neck and upper extremities, hiccough, myoclonic spasms of trunk muscles with terminal generalized weakness and coma, (3) myelitis with paresthesia and paralysis of lower extremities and bladder, the mind remaining clear until the ascending process leads to respiratory failure. Meningeal symptoms have been inconspicuous. A mild lymphocytic pleocytosis in spinal fluid has been

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TREATMENT

There is no specific treatment, and the clinical manifestations of the disease are self-limited However, nonspecific supportive therapy must be utilized in primary infection to preserve life in some instances and in recurrent infection to give symptomatic relief and, in eye infections, to preserve sight.

Primary Infections. SEVERE STOMATITIS may lead to dehydration in infants because of lack of intake This must be combated by appropriate parenteral fluid and electrolyte replacement. Intake of fluid and food must be encouraged, cold semisolids like ice cream are often better tolerated than plain liquids at room temperature Local anesthetics before feeding may be helpful in older children Prevention of secondary bacterial infection may be aided by the frequent use of mouth washes such as Ceepryn 1 4,000 or a freshly prepared suspension of chlortetracycline, 1 to 3 per cent, in 10 per cent glycerine containing 100,000 units of Nystatin per ml This can be used to swab the mouths of infants Systemic penicillin in prophylactic doses may be useful in the younger children where local therapy is difficult

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EPIDEMIOLOGY

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Primary infection Pertinent factors may be classified under 3 heads: (1) *Socio-economic and environmental.* It was early noted that the incidence of infection was considerably higher among persons in a lower than those in a higher socio-economic bracket. The amount of overcrowding and the level of public health education appear to increase and decrease, respectively, the incidence in a given population. (2) *Age. The number of*

Etiology

Appearance and Size. The virus measures about 125 mμ as determined by filtration through gradocol membranes. Under the electronmicroscope, particles appear spherical with a dense center. The early particles have 1 membrane and measure 60 to 100 mμ in diameter, while mature virus has 2 membranes and measures 120 to 180 mμ.

Course of Infection. Reissig and Melnick (1955) have demonstrated that after inoculation of monkey kidney tissue culture the virus disappears from the supernatant medium over the course of 8 hours and becomes detectable again at about the 12th hour. Under the electronmicroscope particles with 1 or 2 membranes appear in both nucleus and cytoplasm. Particles with similar size and structure appear on the surface of the cells at the time infectious virus is detectable in the medium.

Preservation. It can be preserved in 50 per cent glycerol, at −70° C. and by lyophilization.

Antibody Response. The virus has a close antigenic relationship to *Herpesvirus hominis* and a lesser one to *Herpesvirus suis*. An antiserum against *Herpesvirus simiae* neutralizes *Herpesvirus hominis* as well as or better than it neutralizes itself, whereas an antiserum against *Herpesvirus hominis* will not neutralize *Herpesvirus simiae* or only to a very low titer (Burnet et al., 1939; Sabin, 1934).

Diagnosis

An encephalomyelitis developing after contact with monkeys should be considered clinically to be due to this virus until proved otherwise. Isolation of the virus from infected central nervous tissue is the only definite diagnostic criterion. This may be difficult, especially if the patient has lived for some time. Diagnosis can be made, based on a rise in antibody levels in patients who survive for more than 10 days, since a rise of neutralizing antibodies equally against this virus and *Herpesvirus hominis* occurs. The convalescent titer is low against both. In herpetic infection the titer rises, usually to a higher level, only against *Herpesvirus hominis* (Sabin, 1958). The presence of circulating antibodies against *Herpesvirus hominis* does not prevent fatal infection with *Herpesvirus simiae* (Nagler, 1957).

Treatment

In view of the absence of specific therapy, the usual supportive measures employed for a patient with encephalitis assume major importance. It would seem advisable to give steroids in pharmacologic doses for several days as soon as the diagnosis is made. One patient in whom recovery took place began to get better following such therapy (Breen et al., 1958).

Epidemiology

"Normal" monkeys are the reservoir of the virus. Infection can be acquired through bites and apparently by contamination of normal or minimally traumatized skin by monkey saliva or tissues. Contamination of a wound by infected monkey kidney tissue culture cells has resulted in a fatal infection in a man without actual contact with monkeys (Hummeler et al., 1958).

Control Measures

Great care should be taken by those handling monkeys to avoid exposure of naked skin to contact with any secretion or tissue. No vaccine is yet available.

REFERENCES

(For complete references, see also 2nd edition, chapter 23.)

Adair, C. V., Gauld, R. L., and Smadel, J. E. 1953. Aseptic meningitis, a disease of diverse etiology: clinical and etiologic studies on 854 cases, Ann. Int. Med. *39*, 675-704.

Afzelius-Alm, L., 1951, Aseptic encephalo-meningitides in Gothenburg, 1932-1948. Clinical and experimental investigation with special reference to the viruses of herpes, influenza, mumps and lymphocytic choriomeningitis, Acta med. scandinav. (supp. 263) *140*, 1-99.

Anderson, S. G., and Hamilton, J., 1949, Epidemiology of primary herpes simplex infection, M. J. Australia 1, 308-311.

Anderson, S. G., Hamilton, J., and Williams, S., 1950. Attempts to vaccinate against herpes simplex, Australian J. Exp. Biol. & M. Sc. *28*, 579-584.

Andrewes, C. H., 1954, Nomenclature of viruses, Nature, London *173*, 620-621.

Andrewes, C. H., and Carmichael, E. A., 1930, A note on the presence of antibodies to herpes virus in

usual but may be absent; sugar and protein usually have been normal, the pressure has been normal except in the case with the acute encephalitis *The white count has been within* normal limits. Acute abdominal pain, fever and diarrhea have been presenting symptoms in some patients before onset of neurologic signs. Fever has usually but not always characterized the infection. In two bitten patients the local wounds showed redness and swelling with purulent exudate in one and neighboring vesicles in the other The incubation period has been between 10 and 20 days The illness has usually ended in death after a course of 1 day to over 3 weeks from onset of symptoms (Hummeler et al, 1958, Pierce et al, *1958*, Lennette, *1957*, Nagler, *1957*; *Pavilanis, 1957; Sabin, 1949, Sabin and* Wright, 1934) An occasional recovery has been reported but with considerable residual disability *(Breen et al, 1958)*

Pathology

In human material extensive involvement of the brain and the spinal cord has been found The vessels showed perivascular cuffing with mononuclear cells and sometimes necrosis of the walls Necrosis of all elements occurred in some places and the areas appeared demyelinated Satellitosis and neuronophagia were present Intranuclear inclusions were found on occasion in patients dying early in the disease but usually were not observed. The meninges showed vascular engorgement and mononuclear cell infiltration

In the other organs focal necrosis was found in spleen, lymph nodes and adrenals, and cloudy swelling in liver and kidneys In monkeys suffering from a natural primary infection (Keeble *et al*, 1958) *perivascular* cuffing and infiltration with glial cells occurred largely in the roots of the facial and the trigeminal nerves Intranuclear inclusions were found with difficulty in the oligodendrial cells *in the region of perivascular cuffing. Livers* showed varying degrees of damage, from necrosis about the central veins to periportal infiltration of polymorphonuclear and mononuclear cells Intranuclear inclusion bodies were *readily found in relation to the larger* areas of damage. The characteristic tongue ulcers (cf. herpetic ulcers in man) consisted of two zones—a central area of necrosis and a surrounding area of ballooning degeneration; between *these two*, cells with intranuclear inclusion bodies were most readily found, either as single cells or as coencytic masses ("Virus" giant cells). Immediately beyond the area *of ballooned cells the normal epi*thelial cells showed enlargement of the nucleoli

In the rabbit the pathology has been described by Sabin and Hurst (1935). In general the changes resemble those in the monkey. However, when the virus is inoculated intradermally it causes local necrosis and spreads to the central nervous system. This is apparently by way of the peripheral nerves in which an interstitial neuritis occurs.

Experimental Infection, Host Range

This virus is a natural parasite of monkeys in the same way as *Herpesvirus hominis* is a *natural parasite of man. An outbreak of stom*atitis among monkeys due to this virus, entirely comparable with herpetic stomatitis in *man, has been described (Keeble et al, 1958)* The incidence was 2 3 per cent among 1,400 rhesus monkeys In 25 per cent of affected animals there was histologic, even in absence of clinical, involvement of the central nervous system. Antibodies developed on recovery. Among normal monkeys in a colony antibodies have been found in approximately 20 per cent This incidence may rise to 75 to 100 per cent after the animals have been housed together over a period of 6 weeks (Kretch and Lewis, 1954; Keeble et al, 1958; Prier, 1958). The virus can be transmitted readily to rabbits by any route, usually *with a* fatal outcome. Mice are susceptible but only if under 3 weeks of age, in contrast with *Herpesvirus hominis* which can infect older *animals (Sabin, 1958)* 1-day-old chicks have been infected by intracerebral inoculation (Reagan and Bruckner, 1953). It grows readily on the chorio-allantoic membrane of the embryonated hen's egg, producing pocks similar to those of *Herpesvirus hominis*, and egg-adapted strains kill the embryo in 6 days, focal lesions being found in the viscera Rabbit kidney cells are very susceptible and both HeLa and monkey *kidney cells can be in*fected. The cells swell, the nucleoli disappear, the chromatin becomes marginated, and an inclusion body develops; giant cells containing many infected nuclei then begin to appear

as observed in the electron microscope I Herpes simplex virus J Exper Med *100*, 195-202
Munk, K., and Ackermann, W W, 1953, Some properties of herpes simplex virus, J Immunol *71*, 426-430
Nagler, F P O, 1957, Personal communication
Newton, A A, and Stoker, M G P, 1958, Changes in nucleic acid content of HeLa cells infected with herpes virus, Virology *5*, 549-560
Ormsby, H L, 1957, Superficial forms of herpetic keratitis, Am J Ophth *43*, 103-109
Parrott, R H et al, 1954, Clinical and laboratory differentiation between herpangina and infectious (herpetic) gingivostomatitis, Pediatrics *14*, 122-129
Pavilanis, V, 1957, Personal communication
Pelmont, J, and Morgan, H R, 1958, Personal communication
Pierce, E C, Pierce, J D, and Hull, R N, 1958, *B virus*, its current significance to the virologist the description and diagnosis of fatal human infection, Am J Hyg *In press*
Prier, J E, 1958, Personal communication
Pugh, R C B Dudgeon, J A and Bodian M 1955, Kaposi's varicelliform eruption (eczema herpeticum) with typical and atypical visceral necrosis, J Path & Bact *69*, 67-80
Pugh, R C B, Newns, G H and Dudgeon J A, 1954, Hepatic necrosis in disseminated herpes simplex, Arch Dis Childhood *29*, 60-65
Quilligan, J J, and Wilson, J L, 1951, Fatal herpes simplex infection in a newborn infant J Clin & Lab Med *38*, 742-746
Reagan, R L, and Brueckner, A L, 1953, Response of the baby chick to 'B' virus (Strain 1) Am J Trop Med *2*, 864-866
Reissig M, and Melnick J L 1955 The cellular changes produced in tissue cultures by herpes B virus correlated with the concurrent multiplication of the virus J Exper Med *101*, 341-351
Ruchman, I and Dodd K, 1950 Recovery of herpes simplex from blood of a patient with herpetic rhinitis, J Lab & Clin Med *35*, 434-439
Ruchman, I, Welsh A L and Dodd K, 1947, Kaposi's varicelliform eruption Isolation of the virus of herpes simplex from the skin lesions of three adults and one infant Arch Dermat u Syph *56*, 846-863
Sabin, A B, 1934, Studies on *B virus*, Brit J Exper Path *15*, 248-268, 268-279 321-334, 372-380
———, 1949, Fatal *B virus* encephalomyelitis in a physician working with monkeys, J Clin Invest *28*, 808
———, 1958, Personal communication.
———, and Hurst, E W, 1935, Studies on *B virus* IV Histopathology of the experimental disease in rhesus monkeys and rabbits, Brit J Exper Path *16*, 133-148
Sabin, A B, and Wright, A M, 1934, Acute ascending myelitis following a monkey bite, with the isolation of a virus capable of reproducing the disease, J Exper Med *59*, 115-136
Scherer, W F, and Syverton, J T, 1954, The viral range in vitro of a malignant human epithelial cell (strain HeLa, Gey) I Multiplication of herpes simplex, pseudorabies, and vaccinia viruses Am J Path *30*, 1057-1074
Scott, T F McN, 1956, Herpes Simplex—Diagnostic Procedures for Virus and Rickettsial Diseases, pp 313-340, New York Am Pub Health Assoc
———, 1957, Epidemiology of herpetic infections, Am J Ophth *43*, 134-147
Scott, T F McN, Blank, H, Coriell, L L, and Crouse, H, 1950, Pathology and pathogenesis of the cutaneous lesions of variola, vaccinia, herpes simplex, herpes zoster, and varicella in Kidd, J G (ed), The Pathogenesis and Pathology of Viral Diseases, pp 74-99, New York, Columbia.
Scott, T F McN, Burgoon, C F, Coriell, L L, and Blank, H, 1953a, The growth curve of virus of herpes simplex in rabbit corneal cells grown in tissue culture with parallel observations on the development of the intranuclear inclusion body, J Immunol *71*, 385-395
Scott T F McN, Coriell, L L, Blank, H, and Burgoon, C F, 1952, Some comments on herpetic infection in children with special emphasis on unusual clinical manifestations, J Pediatrics *41* 835-843
Scott, T F McN, Coriell, L L, Blank, H, and Gray, A, 1953b The growth curve of the virus of herpes simplex on the chorioallantoic membrane of the embryonated hen's egg, J Immunol *71*, 134-144
Scott, T F McN, Steigman, A J, and Convey J, 1941 Acute infectious gingivostomatitis Etiology epidemiology and clinical picture of a common disorder caused by the virus of herpes simplex. JAMA *117*, 999-1005
Seidenberg, S, 1941 Zur aetiologie der Pustulosis vacciniformis acuta, Schweiz Ztschr Path u Bakt *4* 398-401
Slavin, H B, 1951, The clinical ramifications of infections caused by the virus of herpes simplex, M Clin North America *35*, 563-569
Slavin, H B, and Ferguson, J J, Jr, 1950, Zoster-like eruptions caused by the virus of herpes simplex, Am J Med *8*, 456-467
Slavin, H B, and Gavrette, E, 1946, Primary herpetic vulvovaginitis, Proc Soc Exper Biol & Med *63*, 343-345
Smith, M G, Lennette, E H, and Reames, H R, 1941, Isolation of the virus of herpes simplex and the demonstration of intranuclear inclusion bodies in a case of acute encephalitis, Am J Path *17*, 55-69
Sosa-Martinez, J, Gutierrez-Villegas, L, and Sosa, R M, 1955, Propagation of herpes simplex virus in tissue cultures of rabbit kidney, *J Bact* *70*, 391-399
Steigman A J, and Scott, T F McN, 1947, Evidence that virus of herpes simplex does not cause Vincent's angina of the tonsil, Proc Soc Exper Biol & Med *64*, 244-245
Stoker, M G P, Virus growth and variation To be published in 1959 by Univ Press, Cambridge, Eng
Thygeson, P, and Kimura, S J, 1957, Deep forms of herpetic keratitis, Am J Ophth *43*, 109-113

post-encephalitic and other human sera, Lancet *1*, 857-858

Armstrong, C, 1943, Herpes simplex virus recovered from the spinal fluid of a suspected case of lymphocytic choriomeningitis, Pub Health Rep *58*, 16-21

Behrman, S., and Knight, G, 1954, Herpes Simplex associated with trigeminal neuralgia, Neurology *4*, 525-530

Beveridge, W. I B, and Burnet, F M, 1946, The cultivation of viruses and rickettsiae in the chick embryo, Medical Research Council Special Report Series No 258, p 39, London, Her Majesty's Stat Off

Blank, H, and Brody, M W, 1950, Recurrent herpes simplex A psychiatric and laboratory study Psychosom Med *12*, 254-260

Blank, H, Burgoon, C F, Baldridge, G D, McCarthy, P L, *and Urbach, F, 1951, Cytologic* smears in diagnosis of herpes simplex, herpes zoster, and varicella, J A M A *146*, 1410-1412

Blank, H, Burgoon, C F, Cornell, L L, and Scott, T F McN, 1950, Recurrent aphthous ulcers, Am Med Assn *142*, 125-126

Boake, W C, Dudgeon, J A, and Burnet, MacF, 1951, Recurrent Kaposi's varicelliform eruption in an adult, Lancet *1*, 383-390

Brain, R T, Pugh, R C B, and Dudgeon, J A, 1957, Adrenal necrosis in generalized herpes simplex, Arch Dis Childhood *32*, 120-126, 162

Braley, A E, 1957, Acute herpetic keratoconjunctivitis, *Am J Ophth 43, 105-106*

Breen, G E, Lamb, S G, and Otaki, A T, 1958, Monkey-bite encephalomyelitis Report of a case with recovery, Brit Med J *2, 22-23*

Buddingh, G J, Schrum, D I, Lanier, J C, and Guidry, D J, 1953, Natural history of herpetic infections, Pediatrics *11*, 595-609

Burnet, F M, Lush, D, and Jackson, A V, 1939, The relationship of herpes and B viruses immunological and *epidemiological considerations*, Australian J Exper Biol *17*, 41-51

Burnet, F M, and Williams, S W, 1939, Herpes simplex a new point of view, M J Australia *1*, 637-642

Carton, C A, and Kilbourne, E D, 1952, Activa-

J Bact *59*, 61-68

Crouse, H V, Cornell, L L, Blank, H, and Scott, T F. McN, 1950, Cytochemical studies on the intranuclear inclusion of herpes simplex, J Immunol *65*, 119-128

Dodd, K, Johnston, L M, and Buddingh, G J, 1938, Herpetic stomatitis, J Pediat *12*, 95-102

Dodd, K, and Ruchman, I, 1950, Herpes simplex virus *not the etiologic agent of recurrent stomatitis*, Pediatrics *5*, 883-887

Doerr, R, 1938, Herpes febrilis *in* Doerr, R, and Hallauer, C, vol 1, pp 41-45, Handbuch d Virusforschung, Vienna, Springer.

Elford, W. J, Perdrau, J R, and Smith, W, 1933, The filtration of herpes virus through graded collodian membranes, J. Path & Bact *36*, 49-54

Esteves, J, and Pinto, M R, 1952, Herpetic urethritis, Brit. J Ven Dis *28*, 205-208.

Farmer, E D, 1956, Diseases of the mouth caused by the herpes simplex virus, Proc Roy. Soc Med *49, 640-646*

Findlay, G M., and MacCallum, F O, 1940, Recurrent traumatic herpes, Lancet *1*, 259-261

Gallardo, E, 1943, Primary herpes simplex keratitis Clinical and experimental *study Arch Ophth 30*, 217-220

Gray, A, Tokumaru, T, and Scott, T. F McN., 1958, Different cytopathogenic effects observed in HeLa cells infested with herpes simplex virus, Arch ges Virusforsch *8*, 59-76

Hummeler, K, Davidson, W L, and Henle, W, 1958, Encephalomyelitis due to infection with *Herpesvirus Simiae (B Virus)* A report on 2 fatal cases. *To be published*

Hunt, B P, and Comer, E O, 1955, Herpetic meningoencephalitis accompanying cutaneous Herpes Simplex, Am J Med *19*, 814-823

Jackson, A D M, and Dudgeon, J A, 1951, Kaposi's varicelliform eruption diagnosis and treatment, Great Ormond St J *2*, 125-132

Kaplan, A S, 1957, A study of herpes simplex virus-rabbit kidney cell system by the plaque technique, Virology *4*, 435-457

Kaposi, M, 1895, Pathology and Treatment of Diseases of the Skin, pp 346-347, New York, Wood

Keeble, S A, Christofinis, G J., and Wood, W, 1958, *Natural virus B infection in rhesus monkeys*, J Path & Bact *76*, 189-199

Kilbourne, E D, and Horsfall, F L, Jr, 1951, Studies of herpes simplex virus in newborn mice, J Immunol *67*, 321-329

———, 1951, Primary herpes simplex infection of the adult With a note on the relation of herpes simplex virus to recurrent aphthous stomatitis Arch Int Med *88*, 495-502

Kipping, R H, and Downie, A W, 1948, Generalized infection with the virus of herpes simplex, Brit M J *1*, 247-249

Kretch, U, and Lewis, L J, 1954, Propagation of B virus in tissue cultures, Proc Soc Exper Biol & Med *87*, 174-178

Lazar, M P., 1956, Vaccination for recurrent herpes simplex infection Initiation of a new disease site following the use of unmodified material contain*ing the live virus, Arch Derm 73, 70-71*

Lebrun, J, 1956, Cellular localization of herpes simplex virus by means of fluorescent antibody, Virology *2*, 496-510

Lennette, E H, 1957, Personal communication

Lennette, E H, and van Allen, A, 1957, Laboratory diagnosis of herpetic infection of the eye, Am J Ophth *43*, 118-126

Lynch, F. W, 1945, Kaposi's varicelliform eruption Extensive herpes simplex as a complication of eczema Arch Dermat u Syph *51*, 129-137

Melnick, J. L, and Banker, D D, 1954, Isolation of *B Virus* (Herpes group) from the central nervous system of a rhesus monkey, J. Exper Med *100*, *181-194*

Morgan, C., Ellison, S A., Rose, A M, and Moore, D H, 1954, Structure and development of viruses

as observed in the electron microscope I Herpes simplex virus J Exper Med 100, 195-202
Munk, K, and Ackermann, W W, 1953, Some properties of herpes simplex virus, J Immunol 71, 426-430
Nagler, F P O, 1957, Personal communication
Newton, A A, and Stoker, M G P, 1958, Changes in nucleic acid content of HeLa cells infected with herpes virus, Virology 5, 549-560
Ormsby, H L, 1957, Superficial forms of herpetic keratitis, Am J Ophth 43, 107-109
Parrott, R H et al, 1954, Clinical and laboratory differentiation between herpangina and infectious (herpetic) gingivostomatitis, Pediatrics 14, 122-129
Pavilanis, V, 1957, Personal communication
Pelmont, J, and Morgan, H R, 1955, Personal communication
Pierce, E C, Pierce, J D, and Hull, R N, 1958, B virus, its current significance to the virologist, the description and diagnosis of fatal human infection, Am J Hyg In press
Prier, J E, 1958, Personal communication
Pugh, R C B, Dudgeon, J A, and Bodian, M, 1955, Kaposi's varicelliform eruption (eczema herpeticum) with typical and atypical visceral necrosis, J Path & Bact 69, 67-80
Pugh, R C B, Newns, G H, and Dudgeon J A, 1954, Hepatic necrosis in disseminated herpes simplex, Arch Dis Childhood 29, 60 65
Quilligan, J J, and Wilson, J L, 1951, Fatal herpes simplex infection in a newborn infant, J Clin & Lab Med 38, 742-746
Reagan, R L, and Bruckner, A L, 1953, Response of the baby chick to "B" virus (Strain 1), Am J Trop Med 2, 864-866
Reissig, M, and Melnick, J L, 1955, The cellular changes produced in tissue cultures by herpes B virus correlated with the concurrent multiplication of the virus, J Exper Med 101, 341-353
Ruchman, I, and Dodd, K, 1950, Recovery of herpes simplex from blood of a patient with herpetic rhinitis, J Lab & Clin Med 35, 434-439
Ruchman, I, Welsh, A L and Dodd, K, 1947, Kaposi's varicelliform eruption Isolation of the virus of herpes simplex from the skin lesions of three adults and one infant Arch Dermat u Syph 56, 846-863
Sabin, A B, 1934, Studies on B virus, Brit J Exper Path 15, 248-268, 268-279, 321-334, 372-380
———, 1949, Fatal B virus encephalomyelitis in a physician working with monkeys, J Clin Invest 28, 808
———, 1958, Personal communication
———, and Hurst, E W, 1935, Studies on B virus IV Histopathology of the experimental disease in rhesus monkeys and rabbits, Brit J Exper Path 16, 133-148
Sabin, A B, and Wright, A M, 1934, Acute ascending myelitis following a monkey bite, with the isolation of a virus capable of reproducing the disease, J Exper Med 59, 115-136
Scherer, W F, and Syverton, J T, 1954, The viral range in vitro of a malignant human epithelial cell (strain HeLa, Gey) I Multiplication of herpes simplex, pseudorabies, and vaccinia viruses Am J Path 30, 1057-1074
Scott, T F McN, 1956, Herpes Simplex—Diagnostic Procedures for Virus and Rickettsial Diseases, pp 313-340, New York Am Pub Health Assoc
———, 1957, Epidemiology of herpetic infections, Am J Ophth 43, 134-147
Scott, T F McN, Blank, H, Coriell, L L, and Crouse, H, 1950, Pathology and pathogenesis of the cutaneous lesions of variola, vaccinia, herpes simplex, herpes zoster, and varicella in Kidd, J G (ed), The Pathogenesis and Pathology of Viral Diseases, pp 74-98, New York, Columbia
Scott, T F McN, Burgoon, C F, Coriell, L L, and Blank, H, 1953a, The growth curve of virus of herpes simplex in rabbit corneal cells grown in tissue culture with parallel observations on the development of the intranuclear inclusion body, J Immunol 71, 385-395
Scott, T F McN, Coriell, L L, Blank, H, and Burgoon, C F, 1952, Some comments on herpetic infection in children with special emphasis on unusual clinical manifestations, J Pediatrics 41, 835-843
Scott, T F McN, Coriell, L L, Blank, H, and Gray, A, 1953b, The growth curve of the virus of herpes simplex on the chorioallantoic membrane of the embryonated hen's egg, J Immunol 71, 134-144
Scott, T F McN, Steigman, A J, and Convey, J, 1941, Acute infectious gingivostomatitis Etiology, epidemiology, and clinical picture of a common disorder caused by the virus of herpes simplex J A M A 117, 999-1005
Seidenberg, S, 1941 Zur aetiologie der Pustulosis vacciniformis acuta, Schweiz Ztschr Path u Bakt 4, 398-401
Slavin, H B, 1951, The clinical ramifications of infections caused by the virus of herpes simplex, M Clin North America 35, 563-569
Slavin, H B, and Ferguson, J J, Jr, 1950, Zoster-like eruptions caused by the virus of herpes simplex, Am J Med 8, 456-467
Slavin, H B, and Gavette, E, 1946, Primary herpetic vulvovaginitis, Proc Soc Exper Biol & Med 63, 343-345
Smith, M G, Lennette, E H, and Reames, H R, 1941, Isolation of the virus of herpes simplex and the demonstration of intranuclear inclusion bodies in a case of acute encephalitis, Am J Path 17, 55-69
Sosa-Martinez, J, Gutierrez-Villegas, L, and Sosa R M, 1955, Propagation of herpes simplex virus in tissue cultures of rabbit kidney, J Bact 70, 391-399
Steigman, A J, and Scott, T F McN, 1947, Evidence that virus of herpes simplex does not cause Vincent's angina of the tonsil, Proc Soc Exper Biol & Med 64, 244-245
Stoker, M G P, Virus growth and variation To be published in 1959 by Univ Press, Cambridge, Eng
Thygeson, P, and Kimura, S J, 1957, Deep forms of herpetic keratitis, Am J Ophth 43, 109-113.

Tolentino, P, and de Matteis, F, 1953, Primo-infezione erpetica con epatite a inclusioni nucleari (rilievo biopsica) in bambino di 4 anni, La Clinica Pediatrica *35*, 461-475

Trice, E R, and Shafer, J C, 1953, Recurrent herpes simplex infections of upper extremities with lymphangitis, Arch Derm u. Syph *67*, 37-41

Whitman, L, Wall, M J, and Warren, J, 1946, Herpes simplex encephalitis a report of two fatal cases, J A M A *131*, 1408-1411

Wildy, P, 1955, Recombination with herpes simplex virus, J gen Microbiol *13*, 346-360

Williams, A, and Jack, I, 1955, Hepatic necrosis in neonatal herpes simplex infection, M J Australia *1*, 392-396

Wolf, A, 1950, The pathology of some viral encephalitides *in* Kidd, J G (ed), The Pathogenesis and Pathology of Viral Diseases, pp 194-213, New York, Columbia

Wolman, M, and Bebar, A J, 1952, Cytochemical evidence for the nature of herpes simplex inclusion bodies, J. Infect Dis *91*, 63-68

Womack, C R, and Hunt, B P, 1954, Serologic differences in strains of herpes simplex virus, Science *120*, 227-228

Wood, W, Shimada, F T, 1954, Isolation of strains of virus B from tissue cultures of cynomolgos and Rhesus kidney, Canad J Pub Health *45*, 509

Yoshino, K, 1956, Infection of one-day-old fertile hen's eggs with herpes simplex virus, J. Immunol *76*, 301-307

Yoshino, K, and Taniguchi, H, 1956, Quantitative studies on the interactions between herpes simplex virus and ectodermal cell of chorioallantoic membrane of fertile hen's egg by means of the cover slip infection system, Jap J M Sc. & Biol *9*, 303-319, 321-339

Zuelzer, W W, and Stulberg, C S, 1952, Herpes simplex as the cause of fulminating visceral disease and hepatitis in infancy, A M A Am J Dis Child *83*, 421-439.

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39

Varicella—Herpes Zoster Group

(SYNONYM FOR VARICELLA Chickenpox SYNONYMS FOR HERPES ZOSTER Zona, shingles, zoster)

INTRODUCTION

On the basis chiefly of clinical and epidemiologic data the writer suggested in the previous editions of this book the probability of the identity of viral agents in the etiology of these clinical diseases or syndromes as also had a number of other observers The more recent fundamental studies of Weller (1953) have added sound evidence in the same direction It has become more clearly apparent that these clinical syndromes probably are but 2 phases in the activity of a single virus of uniform antigenic composition without known strain differences, which may be termed the varicella-zoster, or VZ, virus Therefore, in this chapter Varicella and Zoster will be considered as 2 phases of a single disease

Varicella is a mild communicable disease or syndrome confined chiefly to children It is characterized by fever and an itching, vesicular eruption of the skin and the mucous membranes, individual lesions of which are surrounded by erythema

Herpes zoster, which is a severely painful and incapacitating infectious syndrome confined chiefly to adults, is characterized by inflammation of dorsal-root ganglia or extramedullary ganglia of cranial nerves accompanied by crops of vesicles, identical with those of varicella, in areas supplied by affected sensory nerves

HISTORY

The severity of pain and the characteristic localization of both pain and vesicles made the diagnosis and the study of zoster far simpler than varicella, since the latter was generalized and in early medical history was not easily differentiated from smallpox

Zoster was described very early in medical literature and was known to the Greeks as zona Von Barensprung, 1862a, b, 1863, first recorded observations relating the area of skin involved by eruption to its corresponding dorsal-root ganglion In 1884, Landouzy (Levaditi, 1926) on clinical grounds, suggested its infectious nature Head and Campbell, 1900, furnished a classic description of the neuropathology, while Lipschutz, 1921, described the histopathologic changes in the vesicles In 1888, von Bokay, 1909, suggested its possible relation to varicella and later recorded many cases in point, Kundratitz, 1925, inoculated human subjects and apparently obtained some successful takes

Varicella was not described as a clinical entity until the era of Sydenham, it was confused with smallpox until the end of the 18th century Steiner, 1875, experimentally demonstrated it to be infectious. Tyzzer, 1906, described the pathologic response in varicella and provided an excellent picture of the localized lesion Paschen, 1917, described ele-

mentary bodies in the vesicular fluid and suggested a virus as the etiologic agent.

CLINICAL PICTURES

The clinical pictures of varicella and zoster are best understood if varicella is recognized as the primary invasion of an individual by the VZ virus with the resultant generalized *infection but with occasionally a superimposed zosterlike localization of signs* Zoster, occurring separately from varicella, may best be considered as the localized reinvasion by the virus of an immune host or the stimulation to activity of a virus (VZ virus) remaining latent in the human host following its primary invasion as varicella. Thus the localization of zoster could be a reactivation *of the virus latent in the posterior* nerve roots or ganglia by means of an insult such as exposure to cold, pressure, leukemia, or a fresh massive dose of virus Except on rare occasions when a few vesicles erupt over other skin areas, the reactivation or reinvasion remains localized, probably as *the result of a general immunity produced* by the primary varicella invasion

VARICELLA

The incubation period is usually from 14 to 16 days; occasionally, it may be as long as 21 days Approximately 24 hours after the onset of fever, crops of papules, *followed* or accompanied by vesicles with surrounding erythema, appear on the face and the trunk, spreading usually to the mouth, the pharynx and the extremities In chickenpox, the lesions are usually most abundant over the trunk, *while the face and the extremities suffer the most damage from smallpox* Occasionally, the generalized eruption will be accompanied by a localized eruption characteristic of zoster. The eruption occurs simultaneously with the fever, and its duration is proportional to the height and the persistence of the latter. Successive crops of lesions appear, *furnishing all the characteristic stages of* papules, vesicles and crusts at the same time over the affected areas There is a general lymphadenopathy, which is particularly noticeable in the suboccipital and the posterior cervical regions when vesicles occur in the scalp The vesicles, which are indistinguishable from those of zoster, frequently become pustular and the crusts are often removed by *scratching* because of itching. Impetigo, furuncles, septicemia and glomerulonephritis may complicate the picture. Vesicles on the laryngeal mucosa, about the eyes and the genitalia and in the hair present difficult problems The encephalitis, which occasionally complicates varicella, is symptomatically similar to that accompanying zoster but is less common and less severe than that oc*curring in measles Occasionally, neuritis* of the cranial nerves or myelitis may occur. Even temporary blindness may complicate the picture. Small, depressed, white scars may result from severe varicella or a secondary infection of vesicles with pathogenic bacteria No significant changes occur in the number and the character of the blood cells

Pneumonitis occurs in very severe or fatal *cases, particularly in adults, and* radiologically presents a characteristic bilateral nodular infiltration A fatal nodular pneumonitis has been occurring more recently in persons who have contracted varicella while receiving steroid therapy which greatly diminishes resistance, Cheatham et al. (1956)

ZOSTER

The difficulty in establishing an incubation period for zoster may be better understood in the light of this syndrome's apparent status as a reactivation of a localized latent virus (VZ virus) or as a reinvasion of the vi*rus in the posterior nerve root areas,* as mentioned above. Thus the predilection for the posterior nerve root areas may be a quality of viral latency or a tissue susceptibility to reinvasion or both. The author has established from a number of personal observations and more recently from additional observations of a number of clinicians that zoster in adults resulting from exposure to varicella appears usually within 3 to 7 days after exposure to varicella—a reaction closely resembling the accelerated response of a par*tially immune person to vaccinia virus* While the incubation period of zoster secondary to varicella may often be accurately determined, in zoster secondary to tumors or other insults the incubation period obviously would be indeterminable, nor would it be of any significance. Zoste

severe pain, fever, malaise and often exquisite tenderness along the dorsal nerve roots and their corresponding skin areas The pain may be generalized over the area supplied by the nerve or may be sharply localized to the point of nerve exit close to the spine or to the midaxilla where the nerve divides into a deep and a superficial branch

It may be secondary to an insult to a susceptible dorsal nerve root, such as that caused by arsenic, tuberculosis, tumor leukemia, or it may appear without apparent cause At times abortive zoster occurs in which only an erythematous patch appears over the area involved without vesiculation It is rarely bilateral Early in the disease, the lymph node draining the involved area is usually large and tender A crop of papules, rapidly becoming vesicles, appears within 3 or 4 days after onset over the skin supplied by affected nerves and may continue to appear occasionally for 2 or 3 weeks There are also occasionally aberrant vesicles which may be so numerous at times that they simulate the eruption of varicella Vesicles may become pustular if secondarily infected with bacteria As crusts form, pain and tenderness usually disappear Extensive involvement of a ganglion may affect motor roots or it may extend to anterior horn cells, resulting in temporary or permanent paralysis The dorsal roots of the trunk are most frequently involved, while next in frequency are the areas supplied by the 2nd to the 4th cervical ganglia, i e, the shoulders, the arms and the neck Finally, the lower extremities and the perineal area, in that order, are affected least frequently Inflammation of the maxillary division of the trigeminal nerve will produce lesions in the uvula and the tonsillar area, while similar lesions in the anterior part of the tongue, the mouth floor and the buccal mucosa result from involvement of the mandibular division Zoster ophthalmicus follows involvement of the ophthalmic division, which may be particularly serious with resulting scleritis, keratitis and iridocyclitis, ulcers of the cornea and the conjunctiva may occur Vesicles on the pharynx, the tongue, the uvula and the larynx may be extremely painful, laryngeal and faucial paralysis are not infrequent In approximately 50 per cent of cephalic cases of zoster, paralysis occurs When the geniculate ganglion is involved, pain in the auditory canal is followed by a vesicular eruption on the pinna and in the external canal Facial paralysis frequently results, which on rare occasions is permanent Rare involvement of the otic ganglion results in deep pain of the fauces on the affected side, combined with a few vesicles in this area and on the uvula There is usually a mild lymphocytosis in the spinal fluid, unless the number of cells is markedly increased by meningo-encephalitis which is an occasional complication Persistent neuralgia following convalescence in older subjects is common

PATHOLOGIC PICTURE

In contrast with the vesicle of variola which exhibits a reticular degeneration of the prickle-cells, the vesicles of varicella and zoster are obviously the result of a ballooning of the cells with very little reticulation In the early stages, nuclei of infected cells contain the spheroidal and eosinophilic inclusion bodies described by Tyzzer, 1906, in varicella and by Lipschutz, 1921, in zoster In the process of nuclear degeneration, these bodies apparently enter the cytoplasm so that they may be seen simultaneously in nuclei and cytoplasm While in early stages of the development of lesions some proliferation of the epidermis occurs, the corium remains practically unaltered Later, amitoses of the prickle-cells with formation of sickle-shaped, multinuclear cells, the ballooning of cells with their subsequent disintegration, and the entrance of tissue fluids result in the typical vesicle In some large vesicles, the corium may form the base, and in such cases all layers of the skin may be involved, and scarring may result

There are several reports (Johnson, 1940) of young infants having died of varicella A complete postmortem examination was made on one by Johnson (1940), who found characteristic lesions in the esophagus, pancreas, liver, renal pelvis, ureters, bladder and adrenal glands Intranuclear inclusions were present in the endothelium of blood vessels, and at times necrosis of the vessel walls occurred Cellular changes, including intranuclear inclusions, in the visceral lesions were similar to those seen in the skin

Zoster, in addition to skin lesions, produces a characteristic inflammatory reaction in the posterior nerve roots and ganglia Rarely, the

inflammation spreads to the anterior horns, resulting in temporary or permanent paralysis. In the posterior root, there is infiltration of small round cells and red blood cells, necrosis of nerve cells and fibers, and an inflammatory reaction of the ganglion sheath. It has been shown (Ebert, 1949) that zoster can cause destruction of sensory nerve fibers from the corium. The corresponding fibers of the spinal cord undergo degeneration In severe cases, scarring is found in the involved ganglionic area, with loss of cells and nerve fibers and thickening of the ganglion sheath, while in milder cases little or no permanent damage occurs. The dorsal ganglia more often involved, as shown by Head and Campbell, 1900, are those that receive fibers from the viscera through the white ramus of the sympathetic branches

EXPERIMENTAL INFECTION, HOST RANGE

Despite experiments by Eckstein (1933), the results of which indicate that monkeys may be infected by the varicella-zoster virus, there is no conclusive evidence that any host other than man is susceptible. Intranuclear inclusion bodies were found in the tubular cells of monkey testes by Rivers (1926) 5 or 6 days after intratesticular injection of varicella virus in young vervets and green monkeys. Typical varicella lesions in the skin were not produced Steiner, 1875, produced varicella in children by inoculating them experimentally with fluid from varicella vesicles. This work has been fully confirmed by others Zoster vesicular fluid also has induced varicella in experimental human subjects as well as local vesicles more characteristic of *zoster. Evidence of propagation of zoster virus* in human skin grafted on the chorio-allantois of chick embryos was obtained by Goodpasture and Anderson (1944) and Blank et al. (1948), although the chick-embryo tissues themselves appeared to be nonsusceptible. The lesions obtained resembled those of the natural disease with intranuclear inclusions in affected epithelial cells.

More recently Weller (1953) has been able to obtain serial propagation in vitro of the varicella-zoster virus with roller tube cultures of human embryonic skin-muscle tissue and with foreskin tissue obtained from boys between 3 months and 3 years. Both varicella and zoster vesicle fluids were used successfully for the inoculations. Nutrient fluid in the cultures was changed at 3- to 4-day intervals. Typical eosinophilic nuclear inclusions were obtained, and from the focal areas of affected cells peripheral extension of infection to contiguous cells over days or weeks occurred. For subcultures it was necessary to use coarsely ground infected tissue rather than centrifuged fluids from the cultures

ETIOLOGY

Electronmicrographs (Rake et al, 1948; Nagler and Rake, 1948) showed that viral bodies from vesicles of varicella and zoster are, as a rule, brick-shaped and identical in appearance and size (210 to 250 mμ). By the same means it was also demonstrated that zoster viral particles agglutinated in the presence of serum collected from patients convalescent either from zoster or from varicella Vesicles 12 hours old contain numerous viral particles, while those 24 hours old yield very few.

Early laboratory studies, using vesicle fluids and cutaneous crusts as antigens, all suffered from the lack of a virus antigen grown in cultures Nevertheless, a close antigenic relationship was established by the studies of several workers (Rivers and Eldridge, 1929a, b; Amies, 1934) in neutralization, complement-fixation and agglutination tests More recently, the roller tube cultures of virus from both varicella and zoster by Weller (1957) have permitted more critical analysis of the antigens by complement-fixation, neutralization and fluorescent antibody tests (Weller and [illegible] plement fixa-tion [illegible] harve[illegible] *bottle cultures* afforded an excellent antigen. The tests carried out by plate drop method of Fulton and Dumbell (1949), as modified by Svedmyr et al (1952), strongly suggested the identity of varicella and zoster viruses Their identity was also apparent in neutralization tests conducted on virus cultures to which acute and convalescent sera from varicella and zoster patients were added as 10 per cent of the nutrient medium (Weller, 1956). Finally, no detectable antigenic differences were found when cultures of vari-

cella and zoster viruses were prepared on cover slips and treated appropriately with acute and convalescent varicella and zoster sera and then overlayered with antihuman gamma globulin prepared in rabbits and labeled with fluorescein In these tests antibody appeared in zoster and varicella convalescent sera to an almost identical degree By these important methods of antigenic analysis the identity of the viruses of varicella and zoster thus far studied seemed to be fairly well established. The antibodies appear within 4 to 7 days after onset and reach a peak in about 3 weeks with a subsequent slow decline

Also, clinical data on the similarities of the 2 syndromes since von Bokay's original observations show the following relationships

Varicella in children more often induces zoster in exposed adults than it does in exposed children

Zoster, whether in children or adults, rather frequently has been observed as the apparent source of varicella in children (almost never in adults), initiating at times large epidemics The number of such recorded incidents is too great to be explained satisfactorily on the basis of chance alone Varicella and zoster not infrequently occur at the same time in the same child

Certain careful studies, such as those made by The School Epidemics Committee (1938) of Great Britain in a large number of boarding schools, have shown that varicella and zoster occur during the same terms more frequently than is likely to be due to chance and apparently are not seasonal

Despite certain contradictory evidence, zoster occurs frequently in children and adults who previously have had varicella, thus indicating a lack of complete resistance to the virus as a result of previous experience with varicella Zoster and varicella usually produce lasting immunity

Zoster vesicular fluid has been used experimentally to inoculate children intradermally with some success in the production of varicella, Kundratitz, 1925 This has been confirmed (Bruusgaard, 1932) by a second human passage which resulted in a contact infection

In at least 2 isolated island communities, zoster has been observed, whereas no cases of varicella have ever been recorded.

A working hypothesis to relate the above observations may be useful Such a hypothesis could consider the varicella virus as infecting the general population almost universally at an early age A generalized infection with the virus with a relatively long incubation period may permit the development of permanent resistance However, in certain cases, the varicella virus may have neurotropic properties, as indicated by the simultaneous development at times of zoster and varicella, and it may remain within the nerve cells of a few individuals in a manner similar to the symbiosis exhibited by herpes simplex virus and ectodermal cells In adult life, exposure to cold, pressure on a nerve, or a fresh massive dose of varicella virus (in epidemic parotitis, heavy exposure of a resistant individual may at times disturb the parotid without generalized symptoms) may cause a localized virus activity along posterior root fibers with subsequent development of zoster vesicles Zoster produces varicella with greater difficulty and less frequency than does varicella itself This may be explained by the ease of spread of virus from varicella lesions in the mouth and the throat whereas the virus in zoster skin lesions is usually well covered and without a ready means of transport The absence of varicella and the presence of zoster in isolated communities may possibly represent the activity of an endemic neurotropic varicella virus (zoster virus) with which the population has been universally infected but which becomes apparent in relatively few or it may represent the seeding of the virus soon after birth in an inapparent infection

It is still possible, although not at all probable, in view of the newer serologic studies that the viruses of varicella and zoster are distinct entities, possessing certain antigenic constituents in common The growth in tissue cultures and the serologic studies of a greater number of strains of virus should soon resolve this question

DIAGNOSIS

The lesions of herpes simplex may at times be confined to a nerve distribution in the skin, in which case inoculation of vesicular

fluid into suckling mice appears to be the simplest method of differentiation, inasmuch as herpes simplex virus will infect the mice, whereas zoster virus will not. Otherwise, little difficulty should be experienced in differentiating zoster from herpes simplex. Varicella and herpes zoster are frequently indistinguishable. The severe constitutional reaction caused by smallpox with a drop in fever as the rash appears, together with uniformity of development of the eruption in all areas of the skin at the same time and the somewhat larger number of vesicles on the extremities, as compared with the trunk, serve *to distinguish this disease from varicella. Also* in smallpox the lesions tend to be deeper, "shotty" and umbilicated.

TREATMENT

The vesicles of herpes zoster and varicella require similar care to prevent secondary infection, and similar treatment of secondary infections when they occur Oral or parenteral administration of sulfonamides or appropriate antibiotics, and the local application of tyrothricin are useful when the invading bacteria are sensitive to these therapeutic agents Cleanliness of the skin, care of the hands, cutting and cleaning fingernails, and local treatment for relief of irritation and itching and for the prevention of secondary bacterial infections are important measures. In addition, zoster lesions may require sedative ointments, such as 1 per cent cocaine in lanolin. Deep x-ray therapy may also be effective when used early in the disease For late neuralgia, section of sensory roots may be necessary

EPIDEMIOLOGY

20 years, whereas zoster is a disease of extremely low incidence Varicella attacks the sexes equally, *while zoster has a slightly* higher incidence in males, which may be accounted for by the more frequent exposure of older males to severe climatic conditions. Contact infections occur rarely in zoster, while varicella spreads more readily than most infectious diseases, perhaps more readily than measles. Much of the dissemination of varicella is through the air. It may result from the inhalation of small amounts of air by a susceptible person in any enclosed space con-

frequently in the winter and the spring.) Frequency of the recurrence of varicella epidemics depends upon the density of susceptibles; the recurrence rate is lower in rural than in urban populations, acting in this respect in a manner similar to that of measles.

If zoster is an expression of latency of the varicella-zoster virus, as suggested here and in previous editions, as well as by Garland (1943), rather than a reinvasion of virus by infection from an external source, this may well represent, as in herpes febrilis, an example of the immune host becoming an agent of viral spread and viral propagation—an important epidemiologic model for which there must be many others in man.

CONTROL MEASURES

No control measures are available for herpes zoster as it occurs in sporadic form. Convalescent serum is apparently of little, if any, value for protection against varicella, and of no value in its treatment. Transplacental immunity apparently is not present in the newborn. There is available no method of active immunization Under school or institutional conditions, partial disinfection of the air by means of ultraviolet light has limited the epidemic spread of varicella. As a rule, isolation *and quarantine are useless, inasmuch* as the spread of varicella apparently occurs most readily before the disease is clinically manifest. The crusts appear to be infectious while still moist, but dry crusts apparently have lost their infectivity. Thus, if isolation is warranted, 7 days should be sufficient.

REFERENCES

(For complete references, see also 2nd edition, Chapter 27.)

their serological relationship to those of varicella, Brit J Exper Path *15*, 314-320

Blank, H, Coriell, L L, and Scott, T F M, 1948, Human skin grafted upon the chorioallantois of the chick embryo for virus cultivation, Proc Soc Exper Biol & Med *69*, 341-345

Bruusgaard, E, 1932, Mutual relation between zoster and varicella, Brit J Dermat *44*, 1-24

Cantor, [illegible] J, 1921, Herpes and varicella, Brit M J *2*, 508

Cheatham, W J, Weller, T H, Dolan, T F, Jr, and Dower, J C, 1956, Varicella report of two fatal cases with necropsy, virus isolation, and serological studies, Am J Path *32*, 1015-1035

Ebert, M H, 1949, Histologic changes in sensory nerves of the skin in herpes zoster, Arch Dermat & Syph *60*, 641-648

Eckstein, A, 1933, Klinische und experimentelle Untersuchungen zur Frage der Varicellenencephalitis, Ztschr Neurol u Psychiat *149*, 176-190

Fulton, F, and Dumbell, K R, 1949, The serological comparison of strains of influenza virus, J Gen Microbiol *3*, 97-111

Garland, J, 1943, Varicella following exposure to herpes zoster, New England J Med *228*, 336-337

Goodpasture, E W, and Anderson K, 1944, Infection of human skin, grafted on the chorioallantois of chick embryos, with the virus of herpes zoster, Am J Path *20*, 447-455

Johnson, H N, 1940, Visceral lesions associated with varicella, Arch Path *30*, 292-307

Levaditi, C, 1926, L'Herpès et le zona, "ectodermoses neurotropes, étude étiologique et pathogénique, Paris, Masson, 368 pp

Nagler, F P O, and Rake, G, 1948, The use of the electron microscope in diagnosis of variola, vaccinia, and varicella, J Bact *55*, 45 51

Rake, G, Blank, H, Coriell, L L, Nagler, F P O, and Scott, T F M, 1948, The relationship of varicella and herpes zoster electron microscope studies, J Bact *56*, 293-303

Rivers, T M, and Eldridge, L A, Jr, 1929a, Relation of varicella to herpes zoster I Statistical observations J Exper Med *49*, 899-906

———, 1929b, Relation of varicella to herpes zoster II Clinical and experimental observations J Exper Med *49*, 907-917

Svedmyr, A, Enders, J F, and Holloway, A, 1952, Complement fixation with Brunhilde and Lansing poliomyelitis viruses propagated in tissue culture, Proc Soc Exper Biol & Med *79*, 296-300

Great Britain, Medical Research Council, The School Epidemics Committee, 1938 Epidemics in schools, an analysis of the data collected during the first five years of a statistical inquiry, Special Report Series, No *227*, pp *181-184*, London, His Majesty's Stat Off 289 pp

Weller, T H, 1953, Serial propagation *in vitro* of agents producing inclusion bodies derived from varicella and herpes zoster, Proc Soc Exper Biol & Med *83*, 340-346

———, 1956, Problems revealed by the expanding use of tissue culture procedures in studies on infectious agents, Am J Trop Med *5*, 422-429

———, 1958, Observations on the behavior of certain viruses that produce intranuclear inclusion bodies in man, Harvey Lectures, Series *52* (1956-57), 228-254

Weller, T H, and Coons, A H, 1954, Fluorescent antibody studies with agents of varicella and herpes zoster propagated in vitro, Proc Soc Exper Biol & Med *86*, 789-794

Wooller, E J S, 1946, Herpes zoster in an isolated community, Brit M J *1*, 392

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40

Mumps

(SYNONYM: Epidemic parotitis)

INTRODUCTION

Mumps is an acute, self-limiting contagious disease with a high morbidity and a very low mortality. The most constant feature is a painful enlargement of the parotid gland Involvement of other organs is not uncommon, and usually there is a constitutional reaction indicative of a generalized infection

HISTORY

Mumps ■ one of the first diseases which were recognized as clinical entities. In the 5th century B.C., Adams, 1891, its most common manifestations were clearly recorded by Hippocrates, who described it as a mild epidemic sickness characterized by nonsuppurative swellings near the ears and occasionally accompanied by painful enlargement of the testes In modern times, Hamilton, 1790, was among the first to stress orchitis as a frequent manifestation of the infection He also noted certain patients suffering from parotitis who showed symptoms referable to the central nervous system However, it was not until the beginning of the 20th century that clinically apparent and inapparent meningo-encephalitis was generally recognized as a complication. Thereafter it also became evident that other organs, e g, the ovary and the pancreas, might be injured by the infectious agent (Wesselhoeft, 1941). During the first decades of the present century considerable effort was expended in attempts to determine the etiologic agent. Various reports appeared incriminating a variety of bacteria as well as a spirochete, but it was not until 1934 that the causative agent was definitely proved to be a filterable virus (Johnson and Goodpasture, 1934).

CLINICAL PICTURE

Eighteen to 21 days usually elapse between exposure and the development of symptoms, in exceptional cases the period of incubation may be 12 days, or less, or as long as 35 days Infection of the salivary glands is manifested by a varying degree of enlargement. The parotids are those most frequently involved In more than 70 per cent of patients the swelling is bilateral, although one gland may become enlarged first, and after from 1 to 5 days the other may show an increase in size. Pain is usually present when gland enlargement is marked, when such is not the case, pressure on the angle of the jaw may cause pain in the region of the gland. Enlargement of the submaxillary and the sublingual glands is not uncommon and is best determined by palpation. Edematous infiltration of the tissues surrounding the salivary glands is a frequent event and in certain cases may be very marked. Occasionally, presternal edema may be present, a clinical sign that has been recognized only in recent years.

Swelling of a gland usually reaches a maximum within 48 hours and most often persists for 7 to 10 days. Occasionally, some enlargement of the structure still can be discerned after the lapse of several weeks. In the earlier stages a restricted area of redness may be observed immediately about the orifices of the ducts of Stensen and Wharton. Fever of moderate degree may be present 12 to 24 hours before swelling is observed and usually persists from 1 to 3 days. In certain cases it may be absent.

The following is a list of most of the other organs which have been mentioned in the literature as exhibiting signs of involvement: testis, epididymis, prostate, ovary, liver, pancreas, spleen, thyroid, kidneys, labyrinth, eye, thymus, heart (myocardium), vulvovaginal glands, mammary glands (male and female) and nervous system (as manifested by symptoms of encephalitis, encephalomyelitis, neuritis of the facial, trigeminal and optic nerves). In general, it must be emphasized that certain of these structures may be affected not only before or after the parotitis as well as synchronously with it, but even when there is no indication of inflammation of the salivary glands. Furthermore, it should be noted that the frequency with which certain of these organs are attacked has varied widely among several groups that have been studied. Most commonly, the testes or the central nervous system is involved. Orchitis develops on the average once in every 5 cases of parotitis occurring in mature males, although wide variations from this mean may be expected in different outbreaks. In spite of this incidence, sterility resulting from mumps orchitis is rare. This is due in part to the fact that only about one sixth or less of the cases are bilateral and that atrophy of all glandular tissue may not ensue, even in the severe cases (Werner, 1950). An even greater variation in the frequency of meningo-encephalitis, ranging from 0.5 to 10 per cent of the cases of parotitis, has been reported by various observers. There are reports that aseptic lymphocytic meningo-encephalitis unassociated with parotitis but due to mumps virus may occur more frequently in the summer than in the spring (Ritter, 1958). If so, the difficulty of making a clinical diagnosis of mumps meningitis during the summer is increased.

Epididymitis, prostatitis, ovaritis and pancreatitis of marked intensity are noted more rarely. Studies have revealed a regular elevation of serum amylase in patients with simple parotitis as well as parotitis with overt pancreatitis (Zelman, 1944; Applebaum, 1944). An increase in this enzyme does not therefore necessarily indicate pancreatic involvement (Warren, 1955).

The total white blood cell count is variable in different patients, at times being either moderately elevated or depressed and at others within normal limits. The differential count often shows an absolute or relative increase in lymphocytes from the 1st to the 14th day of the illness, but this phenomenon is by no means invariable. Obviously, blood leukocyte counts are of little aid in diagnosis. In contrast with the blood, the white cell count of the spinal fluid is of great significance in the diagnosis of the general syndrome of aseptic lymphocytic meningo-encephalitis, of which mumps virus is one of the known etiologic agents. In mumps encephalitis the number of white cells of the fluid is increased. The total count may range from 8 to 10 cells (normal) to more than 2,000 per cu mm. In one series of 11 cases, the mean count was 434 (Kane and Enders, 1945). In a majority of cases, the proportion of lymphocytes is from 90 to 100 per cent (Kilham, 1949). When determining the significance of an increased white count in the spinal fluid reports should be borne in mind which indicate that in as many as 35 per cent of the cases of simple parotitis presenting no clinical signs of central nervous system involvement the leukocyte count of the cerebrospinal fluid is greater than normal (Bang and Bang, 1943).

Although long suspected on the basis of epidemiologic data, it was not long ago that serologic evidence was obtained which indicates that infection with the virus of mumps may be so innocuous that symptoms do not occur or at least are so mild as to escape observation (Maris et al., 1946). Thus, specific complement-fixing antibody has been found in the blood of one half or more of the tested adults who denied having had mumps (Penttinen et al., 1954). Even in groups of children which have been studied, the number of inapparent infections based on

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40

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tion reaction, chicken or human erythrocytes being used, or the complement-fixation reaction. In suspended fragment cultures of chick amnion, Weller and Enders (1948) demonstrated multiplication of the virus as indicated by the appearance of specific hemagglutinins in the culture fluid. Henle and Deinhardt (1955) showed that cultures of the HeLa strain of human carcinoma cells and of trypsinized monkey kidney can be applied effectively to the isolation and the propagation of the virus. The virus can be detected directly in the culture by its cytopathic effect and by the hemadsorption technic (Shelokov et al., 1958) or by tests for complement-fixing or hemagglutinating antigens in the culture fluid. Virus neutralization tests in vitro can also be used to demonstrate the agent (Deinhardt and Henle, 1956). A comparative study has shown that the tissue culture is more sensitive than the chick embryo in revealing the presence of virus.

ETIOLOGY

Johnson and Goodpasture (1934) showed that the agent passes readily through Berkefeld V and N filters. A considerable range of particle size and shape is indicated by measurements employing graded collodion membranes (Enders and Habel, 1956) and the electronmicroscope (Weil et al., 1948, Dawson and Elford, 1949). Values from 90 to 340 mμ have been reported. Sedimentation diagrams show two boundaries; the principal one had a constant $S = 1311 \times 10^{-13}$ and the secondary an $S = 1940 \times 10^{-13}$ (Weil et al., 1948). Other properties of the virus to which specific references will be found in the review of Enders and Habel (1956) are as follows: Infective virus particles can be easily concentrated by centrifugation (20,000 rpm for 20 minutes), but concentration of soluble complement-fixing antigen requires a greater force and a longer time (30,000 rpm for 1 hour). Most of the properties of the virus are relatively resistant to heat. Complement-fixing antigen withstands 80° C. for 20 minutes. Infectivity and hemagglutinin can be destroyed by 20 minutes at 55° C., but Kilham (1956) has found certain strains with greater stability of both these properties at 56° C. He also demonstrated infectivity and hemagglutinin of some recently isolated strains after 91 days at room temperature. All the properties of mumps virus can be preserved for at least 1 year if kept in skimmed milk at −50° C. to −70° C. The virus has been reported to be most stable between pH 5.8 and 8.0. Its infectivity can be destroyed without impairing hemagglutinin or complement-fixing antigens by exposure to 0.2 per cent formalin at 4° C. for 24 hours. Intense UV radiation for 0.28 seconds will similarly affect the virus. Ether treatment for 30 minutes at 4° C. will destroy infectivity. By treating virus suspended in synthetic mediums with 0.5 per cent beta-propiolactone for 2 hours at pH 7 the infectivity for chick embryos can be destroyed without affecting hemagglutinating capacity (Polley and Guerin, 1957). The substance giving allergic skin tests in hypersensitive individuals can be heated at 65° C. for 20 minutes without loss of activity and is much more resistant to low concentrations of formalin than the infectivity component (Enders, 1943, 1946).

The virus of mumps has the property of causing agglutination of red cells of certain species (Levens and Enders, 1945), causing the lysis of erythrocytes (Morgan et al., 1948) and bringing about fixation of complement in the presence of specific antibody (Enders and Cohen, 1942) and eliciting a delayed allergic reaction in the skin of human beings who have been previously infected (Enders, 1943). The infective, hemagglutinative and hemolytic properties are closely associated. There appear to be two components capable of fixing complement, the "S" (soluble) and the "V" (viral) antigens. These may be separated either by adsorption on red cells (Enders, unpublished data) or by differential centrifugation (Henle et al., 1947, Henle et al., 1948a). Antibodies which neutralize infectivity, inhibit hemagglutination and fix complement appear in the blood serum after infection with the virus. The relationship of the allergenic factor to the other antigenic properties has not been determined, but, like the immediate reaction to vaccinia virus, a positive reaction to it has been shown to be correlated in most instances with an immune state (Enders et al., 1946a; Henle et al., 1951a). The mumps agent has been

this criterion is considerable, at times approaching that of the adults. The available experimental observations indicate that this antibody arises only in response to an infection with the virus or after its inoculation in an inactive state. A more direct indication for the occurrence of inapparent infections is provided by the findings of Henle et al. (1948b), who isolated the agent between the 14th and the 15th days after exposure from the saliva of 6 of 8 volunteers infected experimentally with mumps virus but who failed to develop any clinical signs of infection.

PATHOLOGIC PICTURE

From the diagnostic point of view, pathologic changes induced by the virus are not usually of assistance. In general, the injury is not extensive, with the exception of that occurring in the testis in severe orchitis. The limited observations on material from infected parotid glands indicate that the reaction consists of a serofibrinous exudate with leukocytes in the connective tissue. The cells of the ducts show evidence of degeneration, and necrotic debris and polymorphonuclear cells are found in their lumina (Dopter and Repaci, 1909) Mumps virus has been recovered at autopsy from parotid tissue in which these changes were observed (Weller and Craig, 1949). Characteristic inclusion bodies have not been described in tissues from infected human beings In the testis there are considerable destruction of the epithelium of the seminiferous tubules, marked congestion, punctate hemorrhages and lymphocytic infiltration. Edema and serofibrinous exudation are present in the interstitial tissues (Smith, 1912; Manca, 1932, Gall, 1947). In the pancreas, evidence of congestion, interstitial edema, slight degeneration of the islets, and fat necrosis have been observed The changes induced by the virus in the central nervous system have not been defined, since in the few cases which have terminated fatally the evidence for mumps virus as the cause of death is not conclusive.

Clinical reports suggest that when mumps

vations in developing chick embryos indicate that the virus can cause a variety of deformities (Hamburger and Habel, 1947; Williamson et al., 1957).

Johnson and Goodpasture (1936) reported finding inclusion bodies in the parotids of experimentally infected monkeys. However, Bloch (1937) considered them to be artifacts In spite of their absence in infected living hosts, Brandt (1958) has recently demonstrated the formation of eosinophilic cytoplasmic inclusions in cultures of chick embryo cells in which multiplication of the virus was demonstrated.

EXPERIMENTAL INFECTION; HOST RANGE

In addition to man the animals known at present to be susceptible to virus recovered from naturally infected human beings include certain species of monkeys (Swan and Mawson, 1943) and the chick embryo (Habel, 1945; Levens and Enders, 1945). Guinea pigs (Bolin et al, 1950), suckling mice (Kilham and Murphy, 1952), suckling hamsters (Burr and Nagler, 1953) and suckling white rats (Pospíšil and Brychtová, 1956) have been infected with egg-adapted virus. The presence of complement-fixing antibodies in normal dog sera has been reported (Morris et al, 1956), suggesting that this species may be susceptible. The susceptible species of monkeys include the rhesus (*Macaca mulatta*), the cynomolgus (*Macaca irus*), the pig-tailed (*Macaca nemestrinus*) and the moor (*Macaca maurus*) Typical parotitis characterized by parotid enlargement, facial edema and pathologic changes closely resembling those found in the affected parotid of man can be produced in monkeys only by directly inoculating the gland via Stensen's duct. By means of this procedure the virus can be demonstrated in saliva obtained from patients. Serial passages in monkeys can be effected by injection of a suspension of the parotid gland removed 4 to 7 days following inoculation.

The virus has been isolated by inoculating the amniotic cavity or the yolk sac of the chick embryo (Beveridge et al, 1946). After several passages the virus becomes well adapted to this host and can be recovered from most of its tissues and fluids after an incubation period of 3 or 4 days or longer Tests for the presence of the agent may be made by employing either the hemagglutina-

body against mumps virus develops rapidly in most patients so that by 1 week after the first clinical signs this substance has emerged in a majority. By the end of the 3rd week, in all cases which have been studied, antibody has been demonstrated. In many persons the antibody may persist in the circulating blood for years, although in a concentration lower than those reached during convalescence. In cases where the first specimen of serum has not been obtained sufficiently early to permit the demonstration of a rise in titer, the finding of titers exceeding 1:64 (initial serum dilution) can be interpreted as suggestive of a recent infection. By testing a single specimen of serum taken shortly after onset of symptoms for complement-fixing antibodies against the "S" and the "V" antigens, an early presumptive diagnosis can sometimes be made, since, as Henle et al. (1948a) have shown, early in the disease the level of antibody reacting with "S" antigen is frequently higher than that of antibody reacting with "V" antigen. The antihemagglutinin compared with complement-fixing antibody tends to persist for a longer time at a higher titer. It should be noted that the skin test is of little or no value in diagnosis, since dermal hypersensitivity develops at widely varying intervals following the onset of mumps. Indeed, it is best not to do a skin test for diagnosis, because production of complement-fixing antibody may be stimulated by this procedure in persons who are not suffering from the disease.

Since all strains of mumps virus so far examined appear to belong to a single antigenic type, the demonstration of mumps antibody by any of the technics mentioned above may be taken, with considerable assurance, as evidence of previous infection with this agent. However, one should bear in mind that following infection with mumps virus certain persons develop antibodies which react with Newcastle disease virus of fowls (NDV). In this connection the antigenic cross relationships with influenza D (Sendai) and the Croup-Associated Viruses should also be recalled.

Diagnosis can also be confirmed by recovery of the etiologic agent, for which tissue culture is at the present time the method of choice. However, isolation procedures are usually more laborious than tests for antibodies which are employed as routine in the laboratory diagnosis of mumps. Mumps virus has been recovered in monkey-kidney-cell cultures from saliva, mouth swabs, urine and cerebrospinal fluid. In one study (Utz et al., 1957), 76 per cent of all specimens collected during the first 5 days of illness contained virus, and urine passed as late as the 13th day yielded the agent.

TREATMENT

The treatment of simple parotitis is symptomatic. In severe orchitis, recourse is sometimes had to surgical procedures for reducing the pressure caused by edema. In encephalitis, if the headache is severe, lumbar drainage may afford marked relief. There are no particular procedures employed in the treatment of the other so-called complications of mumps (Wesselhoeft, 1941). Specific therapy, consisting of the administration of various materials derived from human blood, has been attempted in the case of orchitis and meningitis. Most of the efforts have centered upon the prevention and the treatment of orchitis, the materials have been administered before the onset of orchitis but after swelling of the parotid had appeared as well as during the acute stage of orchitic inflammation. They have consisted of whole blood from recent convalescents, convalescent serum, and concentrates of globulin either from pooled normal adult plasma or from pooled convalescent sera. Results obtained in a limited number of patients in which the treatment of established orchitis or meningitis has been attempted have been equivocal, and indeed it might be anticipated that a procedure of this sort would not prove to be efficacious once the virus had caused cellular injury. On the other hand, there are limited data suggesting that concentrated gamma globulin prepared from the plasma of persons recently convalescent from mumps is of some value in preventing the development of orchitis if given after the onset of parotitis. However, concentrated normal gamma globulin has proved to be of no value under these circumstances (Gellis et al., 1945).

Several steroid hormones have been used in attempts to prevent or treat mumps orchitis

classified among the myxovirus group of animal viruses (Andrewes et al., 1955). Serologic cross-reactions have been demonstrated with other members of this group, notably Newcastle disease virus (Evans, 1954) and influenza D virus (De Meio and Walker, 1957) With Chanock's Croup-Associated Agent a similar relationship seems to exist (Chanock, 1956).

Mumps, in nearly all instances, confers a durable immunity, as is indicated by the low rate of second attacks. This rate is usually cited as about 4 per cent, but it is likely that the figure is too high, since it is probable that erroneous diagnoses either of the first or of the second illness have often been recorded Contrary to common belief, when a single parotid is attacked the immunity which ensues would appear on the basis of the available experimental data to be as solid and persistent as when both glands are involved Infection of any organ, even in the absence of parotitis, probably induces the same high order of resistance. Finally, as already noted, there is evidence which shows that clinically inapparent infections, as revealed by the complement-fixation test (Maris et al, 1946) and the skin test for hypersensitivity (Enders et al, 1946a), confer immunity as effectively as does an overt attack. That the active immunity induced by infection can be passively transferred from mother to offspring via the placenta is strongly suggested by the fact that mumps in children under 6 or even 9 months of age is very rare. Furthermore, experiments have shown that complement-fixation and hemagglutination-inhibition antibodies are demonstrable in the cord blood and the venous blood of newborns when present in the mother These antibodies cannot be detected in the blood of most infants 40 to 60 days after birth (Florman and Karelitz, 1953).

A reasonable index of an individual's immunity can be obtained by a determination of his hypersensitivity to skin test antigen, or the level of his complement-fixing, antihemagglutinating or neutralizing antibodies. Because they are easily performed, complement-fixation or skin tests have most often been employed for this purpose. For a review of immunity in mumps see Enders (1946) and Enders and Habel (1956).

DIAGNOSIS

Mumps parotitis can usually be diagnosed with fair accuracy, particularly under epidemic conditions, on the basis of clinical criteria alone The diagnosis of sporadic cases is at times difficult, since a variety of other agents may produce an enlargement of the parotid Thus, it is sometimes necessary to differentiate mumps from suppurative parotitis, enlargements due to foreign bodies in the salivary ducts, neoplasms, Mikulicz's disease, uveoparotid fever and other rare conditions In cases where salivary gland involvement is minimal or absent and in which the virus has attacked other organs, it is frequently impossible to make a conclusive diagnosis on the basis of clinical findings alone. This is especially true in aseptic lymphocytic meningo-encephalitis—a syndrome caused by several viruses and other agents as yet unknown However, it is possible to obtain serologic evidence of any type of infection caused by mumps virus. This can be accomplished (1) by demonstrating the appearance of or increase in antibody capable of fixing complement in the presence of mumps antigen (Enders et al., 1945) and (2) by the demonstration of the appearance or increase of a specific factor, antihemagglutinin (Levens and Enders, 1945; Robbins et al, 1949), in the blood serum which inhibits the agglutination of chicken or human erythrocytes by mumps antigen These procedures have now been studied fairly extensively as diagnostic tools Because of its greater simplicity, the test for antihemagglutinin is to be recommended as routine Antigens for the complement-fixation test may be obtained in the form of a suspension of tissue from an infected parotid gland (monkey), the infected allantoic fluid of the embryonated egg, or the supernatant nutrient fluid of infected tissue cultures (Weller and Enders, 1948; Utz et al, 1957). Material from the infected parotid glands of monkeys is not a satisfactory antigen in hemagglutination-inhibition tests

As with most serologic tests, a definite diagnosis can usually be made only when a significant rise in antibody titer is demonstrated by examining 2 specimens of serum taken at appropriate intervals following the onset of the disease. Complement-fixing anti-

body against mumps virus develops rapidly in most patients so that by 1 week after the first clinical signs this substance has emerged in a majority. By the end of the 3rd week, in all cases which have been studied, antibody has been demonstrated. In many persons the antibody may persist in the circulating blood for years, although in a concentration lower than those reached during convalescence. In cases where the first specimen of serum has not been obtained sufficiently early to permit the demonstration of a rise in titer, the finding of titers exceeding 1:64 (initial serum dilution) can be interpreted as suggestive of a recent infection. By testing a single specimen of serum taken shortly after onset of symptoms for complement-fixing antibodies against the "S" and the "V" antigens, an early presumptive diagnosis can sometimes be made; since, as Henle et al (1948a) have shown, early in the disease the level of antibody reacting with "S" antigen is frequently higher than that of antibody reacting with "V" antigen. The antihemagglutinin compared with complement-fixing antibody tends to persist for a longer time at a higher titer. It should be noted that the skin test is of little or no value in diagnosis, since dermal hypersensitivity develops at widely varying intervals following the onset of mumps. Indeed, it is best not to do a skin test for diagnosis, because production of complement-fixing antibody may be stimulated by this procedure in persons who are not suffering from the disease.

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Diagnosis can also be confirmed by recovery of the etiologic agent, for which tissue culture is at the present time the method of choice. However, isolation procedures are usually more laborious than tests for antibodies which are employed as routine in the laboratory diagnosis of mumps. Mumps virus has been recovered in monkey-kidney-cell cultures from saliva, mouth swabs, urine and cerebrospinal fluid. In one study (Utz et al., 1957), 76 per cent of all specimens collected during the first 5 days of illness contained virus, and urine passed as late as the 13th day yielded the agent.

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Their value is not established, although cortisone may be effective as an analgesic (Smith and Bishir, 1958).

EPIDEMIOLOGY

Mumps is not confined to any area or climate but is world-wide in its distribution. In settled populations, it occurs as an endemic disease throughout the entire year Periodically, however, an increase in the incidence is recorded, usually during the winter and early spring months Although there is a seasonal increase at this time, which may or may not reach epidemic proportions, it is especially marked at intervals of about 7 or 8 years when severe epidemics may be observed. Both sexes are equally susceptible Although predominantly a disease of childhood, adults who have escaped infection as children are often attacked Although among the commonest of infections, a positive history of mumps is obtained less frequently among adults than is the case with certain other diseases of childhood, such as measles (mumps about 60%: measles about 90%) The frequency of inapparent infections in mumps and their rarity in measles would appear to account largely for the difference noted. The only known reservoir of infection is man. Transmission of the virus apparently takes place through direct contact, air-suspended droplets, or fomites contaminated with saliva The recent demonstration of mumps virus in human urine raises the possibility that this is a source of natural infection The exact period during which a patient may be able to transmit the disease has not been determined. There is evidence based on the isolation of virus from naturally and experimentally infected subjects that the period of infectivity may extend from 6 days before to 9 or more days after the appearance of glandular enlargement. Since the virus has been demonstrated in infected individuals who have no salivary gland enlargement, it is probable that they also are able to transmit the disease (Henle et al., 1948b; Kilham, 1949). For reviews of the epidemiology of mumps see the papers by Gordon and Heeren (1940) and Gordon and Kilham (1949)

CONTROL MEASURES

Attempts have been made to prevent the development of mumps (1) by passive immunization through the use of certain of the products mentioned for specific therapy and (2) by active immunization through the injection of inactivated or attenuated mumps virus. Most observers, who have used unconcentrated serum derived from those recently convalescent, have believed that it is of value as a temporary prophylactic measure, provided that it is administered in doses of from 5 to 20 cc. within the first few days following exposure. There is general agreement that any passive immunity so conferred is of short duration, i e., probably 2 or 3 weeks. However, a critical analysis of the available data leaves some doubt as to whether the procedure is sufficiently dependable to be of value. Experimental studies would suggest that these data have been interpreted too optimistically. Thus unpublished data (Janeway and Enders) indicate that very large doses of convalescent sera (200 ml.) might fail to prevent the disease Moreover, the concentration of virus neutralizing antibodies in convalescent sera appears to be relatively low as compared with certain other viral diseases, such as measles

During the last few years work has been undertaken on the development of a practicable method of inducing active immunity. The immunizing effect in monkeys and in human beings of virus present in emlusions of infected parotid glands from monkeys and inactivated by formalin has been studied. Any resistance so induced was challenged by inoculation of virulent virus; the vaccine prevented the development of typical infection in from 50 to 60 per cent of those who received it. Although falling short of an ideal prophylactic agent, formalinized virus has thus been shown to be capable of preventing mumps in certain persons under very severe conditions of exposure (Stokes et al., 1946) Following the development of the technic for cultivating the virus in embryonated eggs; a further step toward a practicable vaccine was taken, since this made available unlimited quantities of material containing the virus Egg virus inactivated by ether or ultraviolet light protected monkeys against experimental infection (Habel, 1946). Experiments on the protective effect of such vaccines in man have shown that the frequency of mumps among control groups exposed to the disease was 3 times that among the vaccinated (Habel,

1951a, 1951b) Since the groups included 2,825 individuals among whom 336 cases of mumps were observed, these results appear to leave little doubt of the prophylactic value of inactivated virus as vaccine, provided that exposure occurs within 4 to 6 weeks after it is administered In 1951, Henle et al., (1951c) found that large doses (4 ml) of egg-grown virus inactivated by formalin or ultraviolet light induced formation of antibodies in all susceptible recipients When naturally exposed 9 to 12 months later, a lower incidence of disease was observed in the vaccinated as compared with a control group Recently obtained unpublished data of Henle (1958) confirm the prophylactic effect of vaccination with inactivated virus Neutralizing antibodies in 90 to 100 per cent of susceptible individuals developed after 2 subcutaneous doses of vaccine given 1 to 4 weeks apart Booster doses were required 6 to 12 months thereafter in order to maintain or increase the antibody levels Study of an outbreak in an institution in which a portion of the children had been vaccinated according to this schedule of dosage indicated that the incidence of mumps was thereby reduced from 30 per cent to 5 6 per cent However, the incidence of inapparent infections in vaccinated and nonvaccinated children was similar The value of vaccination after exposure is unknown, although neutralizing antibodies may appear as soon as 1 week after administration of the first dose

In addition to investigations on the use of inactivated virus as a vaccine, studies on the effect of administering virus attenuated by repeated passage through the embryonated egg have been reported It has been shown that after such treatment the virus loses its ability to produce typical parotitis in the monkey and, with rare exceptions, in man also Such attenuated but active material renders the monkey immune to reinfection with highly virulent virus, and, on the basis of limited data, human beings as well (Enders et al, 1946b, Henle et al, 1951b) In the work just mentioned, the attenuated virus was not inoculated parenterally but introduced either via Stensen's duct or sprayed into the oral cavity Attenuated virus given in this way has been shown to stimulate antibody formation in man, although the concentrations attained did not exceed those recorded in sera of persons receiving inactivated virus Results of experiments in human beings (Henle et al, 1951b) in which high concentrations of attenuated virus were sprayed into the mouth show that up to 93 per cent of the treated individuals responded by the formation of complement-fixing antibodies A few of the children were exposed 3 months after immunization to virulent material None developed mumps These findings encourage further investigation of the method On the basis of general knowledge of immunity in virus diseases, it would be anticipated that the resistance established by a virus strain in which suitable reduction of virulence had been achieved would be more effective and more durable than that resulting from vaccination with inactivated material For detailed treatment of the various aspects of specific prophylaxis, the following references may be consulted Wesselhoeft (1940); Enders (1946), Gellis et al (1945), Lyday (1941), Stokes et al (1946), Enders et al (1946a), Gordon and Kilham (1949)

As previously noted, the available data indicate that a patient may transfer the infection to a susceptible person during a period which extends approximately from 6 days before to 9 days after onset of symptoms (Henle et al, 1948b, Gordon and Kilham, 1949) Although some workers may not entirely approve of it, the standard method of control as now recommended consists in the isolation of a patient until all swelling of affected glands has subsided Recent advances in the knowledge of the duration of infectivity may lead to a modification of this recommendation.

REFERENCES

(For complete references, see also Chapter 28, 2nd edition)

Andrewes, C H, Bang, F B, and Burnet, F M, 1955, A short description of the myxovirus group (influenza and related viruses), Virology 1, 176-184

Applebaum, I L, 1944, Serum amylase in mumps, Ann Int Med 21, 35-43

Bang, H O, and Bang, J, 1943, Involvement of the central nervous system in mumps, Acta med scandinav 113, 487-505

Beveridge, W I B, Lind, P E, and Anderson, S G, 1946, Mumps I Isolation and cultivation of

Their value is not established, although cortisone may be effective as an analgesic (Smith and Bishir, 1958).

EPIDEMIOLOGY

Mumps is not confined to any area or climate but is world-wide in its distribution. In settled populations, it occurs as an endemic disease throughout the entire year Periodically, however, an increase in the incidence is recorded, usually during the winter and early spring months Although there is a seasonal increase at this time, which may or may not reach epidemic proportions, it is especially marked at intervals of about 7 or 8 years when severe epidemics may be observed Both sexes are equally susceptible Although predominantly a disease of childhood, adults who have escaped infection as children are often attacked. Although among the commonest of infections, a positive history of mumps is obtained less frequently among adults than is the case with certain other diseases of childhood, such as measles (mumps about 60%, measles about 90%) The frequency of inapparent infections in mumps and their rarity in measles would appear to account largely for the difference noted. The only known reservoir of infection is man Transmission of the virus apparently takes place through direct contact, air-suspended droplets, or fomites contaminated with saliva. The recent demonstration of mumps virus in human urine raises the possibility that this is a source of natural infection. The exact period during which a patient may be able to transmit the disease has not been determined. There is evidence based on the isolation of virus from naturally and experimentally infected subjects that the period of infectivity may extend from 6 days before to 9 or more days after the appearance of glandular enlarge- [illegible]

[illegible] also are able to transmit the disease (Henle et al, 1948b; Kilham, 1949). For reviews of the epidemiology of mumps see the papers by Gordon and Heeren (1940) and Gordon and Kilham (1949).

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REFERENCES

(For complete references, see also Chapter 25, 2nd edition)

Andrewes, C H, Bang, F B, and Burnet, F M, 1955, A short description of the myxovirus group (influenza and related viruses), Virology 1, 176-184

Applebaum, I L, 1944, Serum amylase in mumps, Ann Int Med 21, 35-43

Bang, H O, and Bang, J, 1943, Involvement of the central nervous system in mumps, Acta med scandinav 113, 487-505

Beveridge, W I B, Lind, P E, and Anderson, S G, 1946, Mumps I Isolation and cultivation of

the virus in the chick embryo Australian J Exper Biol & M Sc *24*, 15-19

Bloch, O, Jr, 1937, Specificity of the lesion of experimental mumps, Am J Path *13*, 939-944.

Bolin, V S, Anderson, J A, and Leymaster, G R, 1950, Experimental intraocular infection of guinea pig with mumps virus, Proc Soc Exper. Biol & Med *75*, 166-170

Brandt, C D, 1958, Inclusion body formation with Newcastle Disease and mumps viruses in cultures of chick embryo cells, Virology *5*, 177-191

Burr, M M, and Nagler, F P, 1953, Mumps infectivity studies in hamsters, Proc Soc Exper Biol [redacted] Med *83*, 714-717.

Chanock, R M, 1956, Association of new type of cytopathogenic myxovirus with infantile croup, J Exper Med *104*, 555-576

Dawson, I M, and Elford, W J, 1949, The investigation of influenza and related viruses in the electron microscope, by [redacted] new technique, J Gen Microbiol *3*, 298-311

Deinhardt, F, and Henle, G, 1956, Determination of neutralization antibodies against mumps virus in HeLa cell cultures, J Immunol *77*, 40-46

De Meio, J L, and Walker, D L, 1957, Demonstration of antigenic relationship between mumps virus and hemagglutinating virus of Japan, J Immunol *78*, 465-471

Dopter and Repaci, G, 1909, Contribution a l'etude anatomo-pathologique des oreillons, Arch méd exp *21*, 533-545

Enders, J F, 1943, Observations on immunity in mumps, Ann Int Med *18*, 1015-1019

———, 1946, Mumps techniques of laboratory diagnosis, tests for susceptibility and experiments on specific prophylaxis, J Pediat *29*, 129-142

Enders, J F, and Cohen, S, 1942, Detection of antibody by complement fixation in sera of man and monkey convalescent from mumps, Proc Soc Exper Biol [redacted] Med *50*, 180-184.

Enders, J F, and Habel, K, 1956, Mumps *in* Diagnostic Procedures for Virus and Rickettsial Diseases, New York, Am Pub Health Assoc, pp 281-312

Enders, J F, Kane, L W, Cohen, S, and Levens, J H, 1945, Immunity in mumps I Experiments with monkeys (Macacus mulatta) The development of complement-fixing antibody following infection and experiments on immunization by means of inactivated virus and convalescent human serum J Exper Med *81*, 93-117

Enders, J F, Kane, L W, Maris, E P, and Stokes, J., Jr, 1946a, Immunity in mumps V The correlation of the presence of dermal hypersensitivity and resistance to mumps J Exper Med *84*, 341-364

Enders, J F, Levens, J H, Stokes, J, Jr, Maris, [redacted] P, and Berenberg, W, 1946b, Attenuation of virulence with retention of antigenicity of mumps virus after passage in the embryonated egg, J Immunol. *54*, 283-291

Evans, A S, 1954, Newcastle disease neutralizing antibody in human sera and its relationship to mumps virus, Am J. Hyg *60*, 204-213

Florman, A L, and Karelitz, S., 1953, The fate of mumps antibodies following their passage through the placenta, J Immunol *71*, 55-57.

Gall, [redacted] A, 1947, The histopathology of acute mumps orchitis, Am J. Path *23*, 637-651.

Gellis, S S, McGuinness, A C, and Peters, M, 1945, A study on the prevention of mumps orchitis by gamma globulin, Am J. M Sc *210*, 661-664

Gordon, J E, and Heeren, R. H, 1940, The epidemiology of mumps, Am J M Sc *200*, 412-428

Gordon, J. [redacted], and Kilham, L, 1949, Ten years in the epidemiology of mumps, Am J. M Sc *218*, 338-359

Habel, K, 1945, Cultivation of mumps virus in the developing chick embryo and its application to studies of immunity to mumps in man, Pub Health Rep *60*, 201-212

———, 1946, Preparation of mumps vaccines and immunization of monkeys against experimental mumps infection, Pub Health Rep *61*, 1655-1664

———, 1951a, Vaccination of human beings against mumps. vaccine administered at the start of an epidemic I Incidence and severity of mumps in vaccinated and control groups Am J Hyg *54*, 295-311

———, 1951b, Vaccination of human beings against mumps vaccine administered at the start of an epidemic II Effect of vaccination upon the epidemic Am J Hyg *54*, 312-318

Hamburger, V, and Habel, K, 1947, Teratogenic and lethal effects of influenza A and mumps viruses on early chick embryos, Proc Soc Exper Biol [redacted] Med *66*, 608-617

Henle, G, Bashe, W J, Jr, Burgoon, J S, Burgoon, C F, Hunt, G R, Jr, and Henle, W., 1951c, Studies on the prevention of mumps III The effect of subcutaneous injection of inactivated mumps virus vaccines J Immunol *66*, 561-577

Henle, G, and Deinhardt, F, 1955, Propagation and primary isolation of mumps virus in tissue culture, Proc Soc Exper Biol & Med *89*, 556-560

Henle, G, Harris, S, and Henle, W, 1948a, The reactivity of various human sera with mumps complement fixation antigens, J Exper Med *88*, 133-147

Henle, G, Henle, W, Burgoon, J S, Bashe, W J, and Stokes, J, Jr, 1951a, Studies on the prevention of mumps I The determination of susceptibility J Immunol *66*, 535-549

Henle, G, Henle, W, and Harris, S, 1947, The serological differentiation of mumps complement fixation antigens, Proc Soc Exper Biol & Med *64*, 290-295

Henle, G, Henle, W, Wendell, [redacted] K, and Rosenberg, P, 1948b, Isolation of mumps virus from human beings with induced apparent or inapparent infections, J Exper Med *88*, 223-232.

Henle, G, Stokes, J, Jr, Burgoon, J S, Bashe, W J, Burgoon, C F, and Henle, W, 1951b, Studies on the prevention of mumps IV. The effect of oral spraying of attenuated active virus J Immunol *66*, 579-594

Henle, W, 1958, Personal communication

Holowach, J , Thurston, D L , Becker, B , 1957, Congenital defects in infants following mumps during pregnancy, a review of the literature and a report of chorioretinitis due to fetal infection, J Pediat 50, 689-694

Johnson, C D, and Goodpasture, E W, 1934, An investigation of the etiology of mumps, J Exper Med 59, 1-20

———, 1936, The histopathology of experimental mumps in the monkey, Macacus rhesus, Am J Path 12, 495-510

Kane, L W, and Enders, J F, 1945, Immunity in mumps III The complement fixation test as an aid in the diagnosis of mumps meningoencephalitis J Exper Med 81, 137-150

Kilham, L, 1949, Mumps meningoencephalitis with and without parotitis, Am J Dis Child 78, 324-333

———, 1956, personal communication cited in Mumps in Procedures for Virus and Rickettsial Diseases, p 294, New York, Am Pub Health Assoc

Kilham, L, and Murphy, H W, 1952, Propagation of mumps virus in suckling mice and in mouse embryo tissue cultures, Proc Soc Exper Biol & Med 80, 495-498

Levens, J H, and Enders, J F, 1945, The hemoagglutinative properties of amniotic fluid from embryonated eggs infected with mumps virus, Science 102, 117-120

Lyday, J H, 1941, An evaluation of convalescent serum in the prevention of mumps, J Pediat 18, 473-475

Manca, C, 1932, Über die Mumpsorchitis, Virchows Arch path Anat 285, 436-443

Maris, E P, Enders, J F, Stokes, J, Jr, and Kane, L W, 1946, Immunity in mumps IV The correlation of the presence of complement-fixing antibody and resistance to mumps in human beings J Exper Med 84, 323-339

Morgan, H R, Enders, J F, and Wagley, P F, 1948, A hemolysin associated with the mumps virus, J Exper Med 88, 503-514

Morris, J A, Blount, R E, McCown, J M, 1956, Natural occurrence in dog serum of antibodies against mumps virus, Cornell Vet 46, 525-531

Penttinen, K, Somer, P, Klemola, E, and Kavalo, E, 1954, Studies of vaccination with formolised mumps virus vaccine in the Finnish defence forces, Ann med exper et biol Fenniae 32, 248-256

Polley, J R, and Guerin, M M, 1957, The use of beta-propiolactone for the preparation of virus vaccines I Selection of reaction conditions Canad J Microbiol 3, 863-870

Pospíšil, L, and Brychtova, J, 1956, Adaptation des Mumps virus auf die weisse Ratte (Wistar), Central bl f Bakt Parasitenk, Infektionskrh u Hyg, Erste Abt, Orig 165, 1-8

Ritter, B S, 1958, Mumps meningoencephalitis in children, J Pediat 52, 424-433

Robbins, F C, Kilham, L, Levens, J H, and Enders, J F, 1949, An evaluation of the test for antihemagglutinins in the diagnosis of infections by the mumps virus, J Immunol 61, 235-242

Shelokov, A, Vogel, J E, and Chi, L, 1958, Hemadsorption (adsorption-hemagglutination) test for viral agents in tissue culture with special reference to influenza, Proc Soc Exper Biol & Med 97, 802-809

Smith, G G, 1912, Two cases of orchitis due to mumps treated by operation, Boston Med & Surg J 167, 313-315

Smith, I M, and Bishir, J W, 1958, Treatment of mumps orchitis with ACTH and cortisone, New England J Med 258, 120-124

Stokes, J, Jr, Enders, J F, Maris, E P, and Kane, L W, 1946, Immunity in mumps VI Experiments on the vaccination of human beings with formolized mumps virus J Exper Med 84, 407-428

Swan, C, and Mawson, J, 1943, Experimental mumps transmission of the disease to monkeys attempts to propagate the virus in developing hens' eggs, M J Australia 1, 411-416

Utz, J P, Kasel, J A, Cramblett, H G, Szwed, C F, and Parrott, R H, 1957, Clinical and laboratory studies of mumps I Laboratory diagnosis by tissue culture technics New England J Med 257, 497-502

Warren, W R, 1955, Serum amylase and lipase in mumps, Am J M Sc 230, 161-168

Weil, M L, Beard, D, Sharp, D G, and Beard, J W, 1948, Purification, pH stability and culture of the mumps virus, J Immunol 60, 561-582

Weller, T H, and Craig, J M, 1949, The isolation of mumps virus at autopsy, Am J Path 25, 1105-1115

Weller, T H, and Enders, J F, 1948, Production of hemagglutinin by mumps and influenza A virus in suspended cell tissue cultures, Proc Soc Exper Biol & Med 69, 124-128

Werner, C A, 1950, Mumps orchitis and testicular atrophy I Occurrence, Ann Int Med 32, 1066-1074

Wesselhoeft, C, 1940, Mumps its glandular and neurologic manifestations in Gordon, J E et al, Virus and Rickettsial Diseases, pp 309-348, Cambridge, Harvard

———, 1941, Mumps in Oxford Loose-Leaf Medicine, pp 489-497, New York, Oxford

———, 1955, Acute infectious diseases in pregnancy, Ann Int Med 42, 555-561

Williamson, A P, Blattner, R J, and Simonsen, L, 1957, Cataracts following mumps virus in early chick embryos, Proc Soc Exper Biol & Med 96, 224-228

Zelman, S, 1944, Blood diastase in mumps and mumps pancreatitis, Am J M Sc 207, 461-464

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41

Infectious Mononucleosis

(SYNONYMS Glandular Fever, *Drusenfieber*, Monocytic Angina, Acute Mononucleosis)

INTRODUCTION

Infectious mononucleosis is an acute or subacute disease of young people, usually characterized by irregular fever, sore throat, lymph node enlargement, abnormal liver function occasionally with jaundice, a characteristic blood picture, and the presence of a heterophil antibody in the serum Although the etiology is unknown, it has long been suspected of being a virus disease

HISTORY

The term *glandular fever* was introduced by Pfeiffer in 1889, although there is some question today as to whether the clinical picture which Pfeiffer had in mind is identical with that of infectious mononucleosis In the United States such a disease was first reported by West (1896) in eastern Ohio The recognition of a monocytosis (or lymphocytosis) in certain acute infections was made by Turk in 1907 and later by Burns who in reporting an epidemic of glandular fever in Baltimore in 1909 called attention to changes in the differential white blood count. The term *infectious mononucleosis* apparently began to be used about 1915 but was first employed in a definitive manner by Sprunt and Evans in 1920, who quoted various cases from the literature together with 6 of their own. A year later Tidy and Morley (1921) in England identified the glandular fever of earlier writers with infectious mononucleosis. And in the United States, Longcope, Downey and McKinley added much to our present knowledge of the clinical picture In 1932 it was found that patients ill with this disease develop antibodies against sheep erythrocytes in a titer often considerably above normal (Paul and Bunnell, 1932). This finding subsequently proved to be useful in diagnosis During the 1940's it became evident that acute hepatitis is a far more common part of the clinical picture than had been suspected previously.

CLINICAL PICTURE

The incubation period is thought to be from 4 to 10 days in duration, although Hoagland (1955) believes it to be much longer The average clinical course lasts from 4 to 20 days or longer, with considerable irregularity as to type and duration. Prominent signs are characterized by generalized lymph node enlargement, mononucleosis and an increased titer of sheep cell agglutinins, yet none of these may be present during the first week of the disease, when it is most desirable to obtain a diagnosis. It is of some value, therefore, to give the clinical manifestations in chronologic order.

Incipient symptoms. The onset is often insidious and marked by nonspecific features, such as fever, headache, malaise, fatigue, sore throat, swollen cervical lymph nodes accompanied by stiffness and soreness of the neck,

leukopenia, chilly sensations and occasionally cough. The temperature curve at this early stage is one which may exhibit steplike increases with rather wide swings. Occasionally, early in its course, the fever may reach high peaks of 104° or 105° F. On the other hand, the temperature curve may be that of sustained fever at somewhat lower levels. In spite of this high fever in the evening, the temperature is often down on the following morning, so that for the first few days of illness the patient may get up and go to school or to work as usual—a fact which often makes it difficult for the physician to decide whether or not he is dealing with someone who is really sick. It may not be until the end of the first week that the diagnosis becomes evident. By this time the nodes may be sufficiently large or tender to attract attention, the blood picture begins to become diagnostic, and about this time the sheep cell agglutinins may have risen to significant titers.

Mid-stage Signs and Symptoms. It is in the second week of illness that signs and symptoms of the full-blown disease usually present themselves. Swelling of cervical lymph nodes and generalized glandular enlargement usually has been recorded in about 50 or 60 per cent of cases, respectively. A characteristic feature of these nodes at this stage is that they are tender. The spleen is enlarged in about 50 per cent of patients. A sore, red and moderately inflamed throat is common. In many of the patients the buccal cavity is also involved with stomatitis, often accompanied by patches on the tonsils and the pharynx where lesions due to Vincent's organisms are often demonstrable. Eye signs are not infrequent, and these include conjunctivitis, pain in or back of the eyes, photophobia and, later, swollen eyelids.

A rash occurs in about 10 to 15 per cent of patients. This eruption is diffuse or blotchy, generally limited to the trunk, and frequently described as morbilliform. It is often difficult to determine whether or not it is a drug rash. It may be preceded or accompanied by an enanthem consisting of petechiae on the soft palate.

Jaundice and Hepatitis. Jaundice is another manifestation present in the first or the second week of the disease, seen in about 5 per cent or less of patients, and is the overt manifestation of hepatitis which is almost universal in this disease. Indeed, liver function tests including serum transaminase determinations may reveal abnormalities in as high as 90 per cent of infectious mononucleosis patients (Cohn and Lidman, 1946; Evans, 1948; Watson et al., 1951, Rennie and Wroblewski, 1957). The suggestion has even been made that such tests might be of considerable diagnostic aid in questionable cases of infectious mononucleosis.

Involvement of the central nervous system, first described by Johansen (1931), now constitutes an uncommon but very important consideration. Such manifestations, which have been reviewed by Bernstein and Wolfe (1950), are said to occur in 1 per cent of the proved cases. The clinical picture has been that of acute serous meningitis, with or without encephalitis and neuritis. Signs and symptoms of such involvement are headache, disturbances of consciousness, nuchal rigidity, convulsions, cranial nerve palsy, motor and sensory disturbances. A peculiar feature of this "complication" is the irregularity in time in which these symptoms appear. They may come on from 1 to 3 weeks after onset of the disease, but, on the other hand, they may be the initial indication of the infection.

Cardiac involvement is as a rule subclinical and benign, although the existence of myocarditis in infectious mononucleosis has been confirmed at autopsy, and acute pericarditis has also been described. Clinically, myocarditis can be demonstrated now by transient and nonspecific electrocardiographic alterations.

Renal involvement also occurs, as is evident from the occasional finding of gross or microscopic hematuria, proteinuria and casts. Thrombocytopenic purpura, acute hemolytic anemia, and hemoglobinuria have been observed occasionally. In one fatal case there were small hemorrhages in the spinal cord, mainly in the posterior horns of the gray matter. Rarely x-ray studies in some patients have revealed pulmonary infiltrations indistinguishable from those of primary atypical pneumonia.

Late Stages, Subacute or Chronic Forms. Usually, the acute disease lasts from 10 days to 2 weeks with symptoms subsiding within 3 weeks, although lymphocytosis may

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41

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The term *glandular fever* was introduced by Pfeiffer in 1889, although there is some question today as to whether the clinical picture which Pfeiffer had in mind is identical with that of infectious mononucleosis In the United States such a disease was first reported by West (1896) in eastern Ohio The recognition of a monocytosis (or lymphocytosis) in certain acute infections was made by Turk in 1907 and later by Burns who in reporting an epidemic of glandular fever in Baltimore in 1909 called attention to changes in the differential white blood count. The term *infectious mononucleosis* apparently began to be used about 1915 but was first employed in a definitive manner by Sprunt and Evans in 1920, who quoted various cases from the literature together with 6 of their own A year later Tidy and Morley (1921) in England identified the glandular fever of earlier writers with infectious mononucleosis. And in the United States, Longcope, Downey and McKinley added much to our present knowledge of the clinical picture. In 1932 it was found that patients ill with this disease develop antibodies against sheep erythrocytes in a titer often considerably above normal (Paul and Bunnell, 1932) This finding subsequently proved to be useful in diagnosis. During the 1940's it became evident that acute hepatitis is a far more common part of the clinical picture than had been suspected previously

CLINICAL PICTURE

The incubation period is thought to be from 4 to 10 days in duration, although Hoagland (1955) believes it to be much longer. The average clinical course lasts from 4 to 20 days or longer, with considerable irregularity as to type and duration Prominent signs are characterized by generalized lymph node enlargement, mononucleosis and an increased titer of sheep cell agglutinins, yet none of these may be present during the first week of the disease, when it is most desirable to obtain a diagnosis. It is of some value, therefore, to give the clinical manifestations in chronologic order.

Incipient symptoms. The onset is often insidious and marked by nonspecific features, such as fever, headache, malaise, fatigue, sore throat, swollen cervical lymph nodes accompanied by stiffness and soreness of the neck,

been inoculated 16 days before with similar material from a patient with infectious mononucleosis. The volunteer later developed hematologic and serologic evidence of the disease, but had no clinical symptoms. Wising, in 1942, used 250 ml of heparinized whole blood from a patient in the acute phase of infectious mononucleosis and injected this intravenously into volunteers, one of whom developed an illness clinically suggestive of infectious mononucleosis, but with only a slight rise in sheep cell titer. Havens in 1945-46 and Evans (1947b, 1950) attempted experimental transmission in more than 40 human volunteers, aged 19 to 25 years, by using pooled human throat washings, and serum and stool preparations inoculated by oral, intranasal, intramuscular and intracutaneous routes. However, although suggestive evidence of mild infectious mononucleosis occurred in several inoculated subjects, there was insufficient evidence to term any of these transmissions truly successful.

ETIOLOGY

A number of different microbial forms, both bacterial and spirochaetal, have been suspected of being the etiologic agent of infectious mononucleosis, but as yet none has been established. The present view is that if the agent is a bacterium or a protozoan, it is a most difficult one to demonstrate. The chances are better that this disease is of viral origin, but here again this has not been demonstrated. The only known properties of this as yet unidentified agent are those which have been mentioned under other headings in this chapter, which deal with transmission experiments. To indicate that this situation needs further investigation is a gross understatement. As to modes of human transmission, the balance of circumstantial evidence suggests that the mouth is the portal of entry, and recently Hoagland (1955) has suggested that, on the basis of clinical records, infectious mononucleosis may well be transmitted by direct and intimate oral contact, which allows for moderate salivary exchange, and that an incubation period of 33 to 49 days follows such exposure. Besides the implication that the agent may be in the mouth, suggestive information has come from the experimental work of Sohier et al. (1940) and Wising (1942) that during the acute phase of the disease the agent is in the blood and the lymph nodes.

DIAGNOSIS

Owing to its extremely variable clinical manifestations, infectious mononucleosis has one or more features in common with a large number of other diseases. In this respect at least, the serologic test is a particularly useful diagnostic measure.

Differential Diagnosis. In the differentiation of infectious mononucleosis from those diseases associated with sore throat and pharyngitis one should be on the lookout for cases of *Vincent's angina, diphtheria, herpetic pharyngitis, aphthous stomatitis* and, more commonly, *acute follicular tonsillitis* with its frequently associated regional lymph node enlargement. Other conditions with lymph node enlargement, particularly enlargement of the cervical chain, include the following: *tuberculosis, Hodgkin's disease, mumps* and occasionally *syphilis*. Certain acute diseases associated with a rash, notably *German measles* and recently described ECHO virus infections, have been confused with infectious mononucleosis. Also in this category is *serum disease* and *an untoward response to drugs*.

From the standpoint of those diseases which present a comparable blood picture, *acute lymphatic* or *monocytic leukemia* may be mistaken for infectious mononucleosis. Bone marrow biopsy may be inconclusive as may be the microscopic appearance of a lymph node removed early in the illness, although in well-established chronic lymphatic leukemia the characteristic features of an invasive process are present. Sheep cell agglutinins are not present in the leukemias.

Of considerable importance is the ease with which infectious mononucleosis may be confused with *infectious hepatitis*. Hepatitis may be present in both conditions, and during the early stages of infectious hepatitis a blood picture not unlike that seen in infectious mononucleosis is common. The serologic test is particularly valuable here, as sheep cell agglutinins do not reach diagnostic titers in infectious hepatitis.

Other diseases which may be confused with

persist for a few weeks, and the lymph nodes may remain somewhat enlarged for months. On the other hand, complete recovery may be delayed for many months. In Niederman's recent (1956) series of 166 patients, about 10 per cent had either objective or subjective symptoms beyond 3 weeks Generalized lymph node enlargement continued in 73 per cent, and splenomegaly in 47 per cent of the individuals Symptoms of malaise and anorexia persisted in some instances for several weeks, and one patient experienced generalized adenopathy and low-grade fever for a period of 12 months The syndrome included: ease of fatigue, exhaustion, aching of the legs, weakness, depression, afternoon elevation of temperature from 99.8° to 101° F, splenomegaly, low blood pressure, and the presence of infectious mononucleosis cells in the blood. This syndrome has been known to last for 4 years or even longer.

Blood Picture. Anemia is uncommon, and the *red blood count* usually remains at normal levels Rarely, however, hemolytic anemia appears as a complication There are wide variations in the *total white blood cell* count, but an early leukopenia is often apparent (i e., between the 4th and the 10th days). The low counts range around 4,500 per cu mm, an extreme low being about 2,000 Leukopenia is then followed by a moderate leukocytosis, averaging about 10,000; it may range as high as 50,000 white blood cells or even greater The leukopenia is a *granulocytopenia*, and actually the granulocytes remain more or less reduced in number for almost 2 weeks. The subsequent increase in total white cells beginning around the 10th to the 17th days is largely due to an increase of lymphoid cells The nature of these characteristic, mononuclear (infectious mononucleosis) cells has been the basis of much discussion It is usually thought that most of them are altered or pathologic lymphocytes. The abnormal lymphocyte is variable in size and staining qualities, although it is generally large with a light-staining cytoplasm A small proportion of these cells, as well as the normal small lymphocytes, may show fenestration in the nucleus

In general, the blood picture in this disease is an exaggeration of the response familiar in a variety of acute febrile diseases of virus origin, particularly reminiscent of that seen in infectious hepatitis.

PATHOLOGIC PICTURE

Although it is suspected that infectious mononucleosis is a respiratory infection, with the mouth as the portal of entry, this has not yet been proved It would seem, in any event, that it becomes a *systemic infection*.

Biopsy Material. Custer and Smith (1948) have studied material from over 100 lymph node biopsies. In the latter series the lymph nodes displayed a variety of appearances ranging from a scattering of abnormal lymphocytes, to the distortion of architectural structure as a result of lymphocytic or reticuloendothelial hyperplasia. Serial liver biopsies have been used to follow the diffuse hepatitis which has a histologic picture close to but not identical with that of infectious hepatitis.

Autopsy Material. Deaths are rare, therefore the amount of this material is not large. In Custer and Smith's autopsy series of 9 cases the *gross* pathologic changes were almost exclusively confined to enlargement of lymphoid tissues, especially the spleen Nasopharyngeal lymphoid hyperplasia was constant Histologic observations revealed more or less generalized lesions widespread throughout the body and consisting of focal or perivascular aggregates of normal and abnormal lymphocytes In some cases reticuloendothelial hyperplasia was so marked as to suggest a lymphoma.

EXPERIMENTAL INFECTION; HOST RANGE

Experimental transmission, except for a few suggestive attempts in man, has not been achieved or confirmed. Repeated attempts to transmit this disease to small laboratory animals and primates, including chimpanzees, have been unsuccessful in almost every instance Such attempts have been made by Bang (1943), Julianelle et al. (1944) and others, in addition, inoculations of embryonated eggs and more recently a variety of tissue culture systems have been tried, all unsuccessfully.

The work in man deserves more than passing mention. In 1940, Sohier et al. inoculated a human volunteer intramuscularly with 6 ml of whole blood from a monkey which had

this test is enhanced when sequential tests are used.

The presence of a positive Kahn, Wassermann or Mazzini test has been noted in about 5 per cent of the cases. Usually this has been seen in those cases which have had high titers of sheep cell agglutinins and, as the agglutinins have regressed, so also have the titers of the Wassermann and the Kahn tests

TREATMENT

Acute Stage. Patients may be cared for by a general practitioner and seldom require the supervision of specialists Hospitalization is not an essential part of the therapy, but many patients with this disease are sick enough to require hospital care Isolation technic is not necessarily recommended Therapy consists mainly in caring symptomatically for a patient who may have high fever (105° F), severe stomatitis, sore throat and painful swollen lymph nodes, particularly in the neck Always there is the danger of rupture of the spleen Bed rest, adequate fluid intake and light diet as for any acute infection are indicated

The care of the mouth and irrigation of the throat with warm saline solutions are particularly indicated If stomatitis is present, the use of potassium perborate, or in some instances potassium chlorate solution (a saturated solution diluted 50% in water) as a mouth wash not more than twice a day, is helpful

It is the physician's duty to be on the alert for complications, such as jaundice, or rarely, meningo-encephalitis and hemolytic anemia In the cases with hepatitis the same general principles of therapy used in infectious hepatitis should be considered There is no particular reason to reduce the amount of fat in the diet after the patient is on the mend

Although the disease is rarely fatal, one should understand that fatalities are not unknown and when they occur are often the result of rupture of the spleen This may result from minimal trauma Therefore, repeated abdominal palpitation and exertion on the part of the patient while the spleen is enlarged should be avoided Exploratory laparotomy is indicated for severe abdominal pain in this disease, for it may be a lifesaving procedure

Chlortetracycline has been suggested, but controlled studies do not indicate that this antibiotic is effective Sulfonamide drugs are

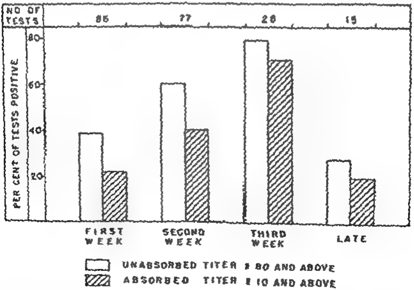

FIG 119 Heterophil antibody determinations in a series of 166 cases of infectious mononucleosis listed according to various stages of the disease (Niederman, J C, 1956, Yale J Biol & Med 28, 629)

infectious mononucleosis include. *acute appendicitis, trichinosis, acute rheumatic fever, undulant fever* and *acute infections of the central nervous system*, particularly the group known as *aseptic meningitis*

Acquired *toxoplasmosis*, as demonstrated by Siim (1951) in Denmark and Skipper et al (1954) in England, produces in a certain number of young adult patients, a febrile illness associated with lymphadenopathy. The diagnosis of toxoplasmosis is made by special serologic means.

It is quite possible that a clinical picture resembling that of infectious mononucleosis, as we know it today, embraces a number of different entities, some of them ill-defined, including a disease described by Blake et al. (1942). In none of these, however, is the infectious mononucleosis (heterophil) antibody present

Another condition to be considered here, which is usually but not always confined to children, is that of *acute infectious lymphocytosis*, described by Smith (1944) and Barnes et al (1949).

Perhaps the most valuable of laboratory tests are the total white and differential blood counts to which reference has already been made. Recognition of a lymphocytosis and the predominating type of mononuclear cells which have been designated as characteristic of infectious mononucleosis is of primary importance However, the mere presence of these cells, *in the absence of other typical signs*, is not diagnostic. Randolph and Hettig (1945) have reported the fairly frequent occurrence of these infectious mononuclear cells in the peripheral blood of individuals with a variety of allergic conditions.

Serologic Diagnosis. The sheep cell or heterophil agglutination test for the diagnosis of infectious mononucleosis is a *nonspecific* [illegible] which [illegible] nce of [illegible] he infectious mononucleosis antibody, which differs from other antibodies found in human [illegible] these [illegible] differentiation between these various antibodies is based on the fact that sheep cell agglutinins in infectious mononucleosis can be completely absorbed by beef erythrocytes but are not reduced significantly by absorption with guinea pig kidney. In serum sickness, sheep cell agglutinins can be completely absorbed by both guinea pig kidney and beef erythrocytes. Sheep cell agglutinins in normal serum are not ordinarily absorbed by beef cells but can be completely or almost completely absorbed by guinea pig kidney.

For the development of technics useful in carrying out this test the reader is referred to Stuart et al. (1936), Davidsohn (1937), Paul (1949); and, for a qualitative, rapid micromethod to Evans (1947a). There is some disagreement concerning the arbitrary figure which has been selected as representing a diagnostic titer of sheep cell agglutinins in infectious mononucleosis serum This stems from the fact that variable technics have been used When the procedure recommended by the author in 1949 is followed, titers which range from 1:10 to 1 40 should be considered negative, from 1:80 to 1:160 as suspicious; and those above 1:160 may be referred to as *positive. The best criterion in the early stages* is that of a rising titer. The values quoted above apply to an unabsorbed titer, but it should be recognized that any completely absorbed titer with guinea pig kidney of 1·10 or greater is serologically valid for the diagnosis of infectious mononucleosis

Although useful as a diagnostic test, the heterophil antibody test is not always positive in infectious mononucleosis; In fact, figures on this point differ widely in different series There are also many examples of false positives, Zarafonetis et al. (1953). The degree to which *positive titers*, obtained according to criteria just described, were recorded in various weeks of the disease in a series of 166 cases is illustrated in Figure 119. These data are based on the results of 206 serologic tests It will be seen that during the first week of illness, only 38 per cent of the cases had significantly positive titers, but that this increased to 60 per cent during the 2nd week of the disease. A precipitous decline in the heterophil antibody titers then follows in the 4th and subsequent weeks, and from this it is evident that high titers are apt to be transient and short-lived. It is also apparent from these data that the diagnostic value of

lower peak in the fall, perhaps associated with the date of the opening of schools or colleges In other series, no seasonal trend has been observed

Little is known about *immunity* in this disease, although it is strongly suspected that one attack gives lasting immunity. Evidence for this rests primarily upon the age distribution of the cases, which [illegible] seen in individuals over 35 is that the majority of them have already had it *On the other hand, there* are not infrequent claims of second attacks separated by several years It is quite probable that they may occur, although carefully *documented second attacks are not often seen.*

In general, therefore, one can assume that this disease is spread by some form of person-to-person association, and, either that it is not due to a highly infectious agent, or that a special type of intimate contact is necessary It would also seem that the degree of resistance (immunity) in most adult populations is moderately high

REFERENCES

Bang, J, 1943, Experiments with the transmission of infectious mononucleosis to man, Acta med scandinav *113*, 304-310

Barnes, G R, Yannet, H, and Lieberman, R, 1949 A clinical study of an institutional outbreak of acute infectious lymphocytosis Am J M Sc *218*, 646-654

Bernstein, A, 1940, Infectious mononucleosis, Medicine 19, 85-159

Bernstein, T C, and Wolff, H G, 1950, *Involvement of the nervous system in infectious mononucleosis*, Ann Int Med *33*, 1120-1138

Blake, F G, Weir, L G, and Craige, B, 1942, An apparently undescribed infectious exanthem Yale J Biol & Med *14*, 573-579

Cohn, C, and Lidman, B I, 1946, *Hepatitis without jaundice in infectious mononucleosis*, J Clin Invest *25*, 145-151

Custer, R P, and Smith E B, 1948, The pathology of infectious mononucleosis, Blood, *3*, 830-857

Davidsohn, I, 1937, Serologic diagnosis of infectious mononucleosis, J.A.M.A *108*, 289-295

Evans, A S, 1947a, A simplified "qualitative" method for heterophile antibody determination using capillary blood and a white cell pipette, J Lab & Clin Med *32*, 1278-1281

———, 1947b, Experimental attempts to transmit infectious mononucleosis to man, Yale J Biol & Med *20*, 19-26

———, *1948, Liver involvement in infectious mononucleosis*, J Clin Invest *27*, 106-110

———, 1950, Further experimental attempts to transmit infectious mononucleosis to man, J. Clin Invest *29*, 508-512

Evans, A S., and Robinton, E, 1950, An *epidemiological study of infectious mononucleosis* in a New England College, New England J Med *242*, 492-496

Evans, W F., and Graybiel, A., 1946, Electrocardiographic evidence of cardiac complications in infectious mononucleosis, Am J M Sc *211*, 220-226

Havens, W P, 1945-1946, Unpublished experiments carried *on in the laboratory of the Section of Preventive* Medicine, Yale University School of Medicine

Hoagland, R J, 1952, Infectious mononucleosis, Am J Med *13*, 158-171

———, 1955, The transmission of *infectious mononucleosis*, Am J M Sc *229*, 262-272

Johansen, A H, 1931, Serous meningitis and infectious mononucleosis, Acta *med* scandinav *76*, 269-272

Julianelle, L A, Bierbaum, O S, and Moore, C V, 1944, Studies on infectious mononucleosis, Ann Int Med *20*, 281-292

Leibowitz, S, 1953, Infectious Mononucleosis, Modern *Medical Monographs No 5, New York,* Grune

Longcope, W T, 1922, *Infectious mononucleosis* (glandular fever) with a report of cases, Am J M Sc *164*, 781-808

Niederman, J C, 1956, Infectious mononucleosis at the Yale-New Haven Medical Center 1946-55, Yale J Biol & Med *28*, 629-643

Paul, J R, 1939, Infectious mononucleosis, Bull New York Acad Med *15*, 43-55

———, 1949, The *diagnosis of infectious mononucleosis in* Horsfall, F L (ed.), Diagnosis of Viral and Rickettsial Infections, pp *109-116*, New York, Columbia

Paul, J R, and Bunnell, W W, 1932, The presence of heterophile antibodies in infectious mononucleosis, Am J M Sc *183*, 90-104

Pfeiffer, E, 1889, Drusenfieber, Jahrb f Kinderh *29*, 257-264

Randolph, T G, and Hettig, R A, 1945, Coincidence of allergic disease, unexplained fatigue and lymphadenopathy, possible diagnostic confusion with infectious mononucleosis, Am J M Sc *209*, 306 314

Rennie, *L E*, and Wroblewski, *F*, 1957, The clinical significance of serum transaminase *in* infectious mononucleosis complicated by hepatitis, New England J Med *257*, 547-553

Riley, H D, 1953, Acute infectious lymphocytosis report of two cases in the same family, New England J Med *248*, 92-95

Rusg-Gunn, M A, 1954, Infectious *mononucleosis* a review of the condition as seen in the Royal Navy, Proc *Roy* Soc Med *47*, 759-774

Schultz, A. L., and Hall, W, 1953, Clinical *observations* in 100 cases of *infectious mononucleosis* and results of treatment with penicillin and aureomycin, Ann Int Med *36*, 1498-1512.

Sam, J C, 1951, Acquired toxoplasmosis report of 7 cases with strongly positive serologic reactions, J.A.M.A *147*, 1641-1645

TABLE 31. PERCENTILE AGE DISTRIBUTION BY 5-YEAR AGE-GROUPS OF 2 SERIES OF CASES (1 EUROPEAN AND 1 AMERICAN) OF INFECTIOUS MONONUCLEOSIS

AUTHOR	AREA	NO IN SERIES	AGE GROUPS (IN YEARS)							
			0-5	6-10	11-15	16-20	21-25	26-30	31-35	36+
Thomsen (1942)	Denmark	545	5.5	9.9	10.5	41.0	24.9	5.5	2.5	0.4
Niederman (1956)	New Haven, Conn., USA	166	12.0	4.2	2.4	18.6	38.3	13.2	4.2	6.6

of no value and may be harmful. Steroid therapy is beneficial in selected patients with severe toxic symptoms, particularly of the anginose type.

Subacute Stage. Occasionally, cases of infectious mononucleosis may be extremely protracted, lasting weeks or even months For this reason a guarded convalescence is part of the therapeutic regimen Cases have been reported which have recurred after surgery, the suggestion being that the operation has *precipitated a relapse*

Prophylaxis. There are no measures to be recommended which are known to control the spread of this disease Although isolation technics have been used in some countries for the care of acutely ill patients with this disease and may be theoretically advisable, they are not usually practiced Nevertheless, disinfection of nose and throat discharges would seem to be indicated in the general care of patients with the acute disease

The use of gamma globulin as a prophylactic measure has not been established.

EPIDEMIOLOGY

Increasing in recognition, infectious mononucleosis is now common in many parts of the world, notably the United States, England and Europe A report from the British Royal Navy (Rugg-Gunn, 1954) ranks it second in frequency to rubella, if the common cold and influenza are excluded However, there has been a significant lack of the occurrence of recognizable epidemics of bona fide examples of the disease. Apparently this [illegible] ted easily, for although

Robinton, 1950, Hoagland, 1952). Cross-infections in an open hospital ward have also been notable by their absence. Nevertheless, occasional epidemics have been reported since the 1890's in boarding schools, colleges, foundling homes, hospital wards, prisons and small communities.

As the age distribution of any disease can often be an indication of the most or *least* susceptible segment of the population, or that segment which is more or less heavily exposed, this deserves some attention. In the *majority of series there is an increase in frequency of cases in the age group of 15 to 25 with a concentration of cases in the age group 16 to 20 in one European series, and in the age group 21 to 25 in a series from* the United States (see Table 31). This table demonstrates a rather unusual age distribution for an infectious disease. A number of reports stress the frequency of infectious mononucleosis in childhood, but this has not been the experience of all general hospitals It is possible that, as in the case of infectious hepatitis, infectious mononucleosis may occur far more commonly in childhood than is suspected, but that it is so mild in childhood *that it escapes recognition* As to the *sex distribution,* the disease is more common in males than in females, and from the occupational standpoint it is prone to appear among young people who live in institutions, colleges, or military establishments. Among [illegible] dents than about the United States

those who work in hospitals Indeed, the frequency with which infectious mononucleosis has attacked nurses, interns and medical students is proverbial.

As to *seasonal trends,* opinions differ on this point. In some areas a rise of incidence in the spring has been reported with another

lower peak in the fall, perhaps associated with the date of the opening of schools or colleges In other series, no seasonal trend has been observed

Little is known about *immunity* in this disease, although it is strongly suspected that one attack gives lasting immunity Evidence for this rests primarily upon the age distribution of the cases, which indicates that it is essentially a disease of children and young adults, one could merely infer by deduction that the reason it is seldom seen in individuals over 35 is that the majority of them have already had it. On the other hand, there are not infrequent claims of second attacks separated by several years It is quite probable that they may occur, although carefully documented second attacks are not often seen

In general, therefore, one can assume that this disease is spread by some form of person-to-person association, and, either that it is not due to a highly infectious agent, or that a special type of intimate contact is necessary It would also seem that the degree of resistance (immunity) in most adult populations is moderately high

REFERENCES

Bang, J, 1943, Experiments with the transmission of infectious mononucleosis to man, Acta med scandinav 113, 304-310

Barnes, G R, Yannet, H., and Lieberman, R, 1949, A clinical study of an institutional outbreak of acute infectious lymphocytosis, Am J M Sc 218, 646-654

Bernstein, A, 1940, Infectious mononucleosis, Medicine 19, 85-159

Bernstein, T C, and Wolfe, H G, 1950, Involvement of the nervous system in infectious mononucleosis, Ann Int Med 33, 1120-1138

Blake, F G, Welt, L G, and Craige, B, 1942, An apparently undescribed infectious exanthem, Yale J Biol & Med 14, 573-579

Cohn, C, and Lidman, B I, 1946, Hepatitis without jaundice in infectious mononucleosis, J Clin Invest 25, 145-151

Custer, R P, and Smith, E B, 1948, The pathology of infectious mononucleosis, Blood, 3, 830-857

Davidsohn, I, 1937, Serologic diagnosis of infectious mononucleosis, J A M A 108, 289-295

Evans, A S, 1947a, A simplified "qualitative" method for heterophile antibody determination using capillary blood and a white cell pipette, J Lab & Clin Med 32, 1278-1281

———, 1947b, Experimental attempts to transmit infectious mononucleosis to man, Yale J Biol & Med 20, 19-26

———, 1948, Liver involvement in infectious mononucleosis, J Clin Invest 27, 106-110

———, 1950, Further experimental attempts to transmit infectious mononucleosis to man, J Clin Invest 29, 508-512

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———, 1955, The transmission of infectious mononucleosis, Am J M Sc 229, 262-272

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Julianelle, L A, Bierbaum, O S, and Moore, C V, 1944, Studies on infectious mononucleosis, Ann. Int Med 20, 281-292

Leibowitz, S, 1953, Infectious Mononucleosis, Modern Medical Monographs No 5, New York, Grune

Longcope, W T, 1922, Infectious mononucleosis (glandular fever) with a report of cases, Am J M Sc 164, 781-808

Niederman, J C, 1956, Infectious mononucleosis at the Yale New Haven Medical Center 1946-55, Yale J Biol & Med 28, 629-643

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———, 1949, The diagnosis of infectious mononucleosis in Horsfall, F L (ed), Diagnosis of Viral and Rickettsial Infections, pp 108-116, New York, Columbia

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Pfeiffer, E, 1889, Drusenfieber, Jahrb f Kinderh 29, 257-264

Randolph, T G, and Hettig, R A, 1945, Coincidence of allergic disease, unexplained fatigue and lymphadenopathy, possible diagnostic confusion with infectious mononucleosis, Am J M Sc 209, 306-314

Rennie, L E, and Wroblewski, F, 1957, The clinical significance of serum transaminase in infectious mononucleosis complicated by hepatitis, New England J Med 257, 547-553.

Riley, H D, 1953, Acute infectious lymphocytosis report of two cases in the same family, New England J Med 248, 92 95

Rugg-Gunn, M A, 1954, Infectious mononucleosis a review of the condition as seen in the Royal Navy, Proc Roy Soc Med 47, 759-774

Schultz, A L, and Hall, W, 1955, Clinical observations in 100 cases of infectious mononucleosis and results of treatment with penicillin and aureomycin, Ann Int. Med 36, 1498-1512

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Increasing in recognition, infectious mononucleosis is now common in many parts of the world, notably the United States, England and Europe A report from the British Royal Navy (Rugg-Gunn, 1954) ranks it second in frequency to rubella, if the common cold and influenza are excluded However, there has been a significant lack of the occurrence of recognizable epidemics of bona fide examples of the disease Apparently this disease is not transmitted easily, for, although sporadic cases are often prevalent among hospital, university and institutional population groups, infectious mononucleosis rarely has been observed among roommates (Evans and Robinton, 1950; Hoagland, 1952). Cross-infections in an open hospital ward have also been notable by their absence. Nevertheless, occasional epidemics have been reported since the 1890's in boarding schools, colleges, foundling homes, hospital wards, prisons and small communities

As the age distribution of any disease can often be an indication of the most or least susceptible segment of the population, or that segment which is more or less heavily exposed, this deserves some attention. In the majority of series there is an increase in frequency of cases in the age group of 15 to 25 with a concentration of cases in the age group 16 to 20 in one European series, and in the age group 21 to 25 in a series from the United States (see Table 31). This table demonstrates a rather unusual age distribution for an infectious disease A number of reports stress the frequency of infectious mononucleosis in childhood, but this has not been the experience of all general hospitals It is possible that, as in the case of infectious hepatitis, infectious mononucleosis may occur far more commonly in childhood than is suspected, but that it is so mild in childhood that it escapes recognition. As to the *sex distribution*, the disease is more common in males than in females, and from the occupational standpoint it is prone to appear among young people who live in institutions, colleges, or military establishments Among college students throughout the United States this disease has become in recent years a most important medical problem Particularly is it common in medical institutions or among those who work in hospitals. Indeed, the frequency with which infectious mononucleosis has attacked nurses, interns and medical students is proverbial.

As to *seasonal trends*, opinions differ on this point. In some areas a rise of incidence in the spring has been reported with another

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42

The Typhus Fevers

The typhus fevers comprise the first group among the infections which are referred to as the rickettsial diseases of man These infections have several features in common The causative micro-organisms, called rickettsiae, can be observed readily in the light microscope as pleomorphic coccobacillary forms, but they multiply only within certain cells of susceptible species For these reasons the rickettsiae are classified as a family between the viruses and the bacteria A distinctive characteristic of the rickettsiae is their occurrence in various arthropods under natural conditions The rickettsial diseases are divided into 4 main groups on the basis of their clinical features, their epidemiologic aspects and their immunologic characteristics

1 Typhus group (discussed in this chapter)
   A Epidemic (louse-borne) typhus
   B Brill-Zinsser disease (recrudescent typhus)
   C Murine (flea-borne) typhus
2 Spotted-fever group (Chap 43)
3 Scrub typhus (Tsutsugamushi disease) (Chap 44)
4 Q fever (Chap 45)

Trench fever, presumed to be rickettsial in etiology (Chap 46)

EPIDEMIC LOUSE-BORNE TYPHUS

(SYNONYMS Jail fever, war fever, famine fever, morbus hungaricus, *typhus historique, typhus exanthematique, dermotypho, tabardillo, typhus exantematico, Fleckfieber*)

INTRODUCTION

Typhus fever is an acute infectious disease characterized by sustained high fever, severe headache, generalized macular or maculopapular rash, and termination by rapid lysis in 14 to 18 days The over-all case-fatality rate in epidemics is about 20 per cent The etiologic agent was named *Rickettsia prowazeki* in honor of two investigators, Dr Howard Taylor Ricketts, an American, and Dr S von Prowazek, an Austrian, who died of typhus fever in the course of their studies of its etiology (da Rocha-Lima, 1916)

HISTORY

Typhus fever has probably afflicted mankind since ancient times The description by Fracastorius in 1546 is the earliest medical record which is sufficiently clear to identify typhus fever as a separate entity Arguments have been advanced (MacArthur, 1954) that the great epidemic in Athens in 430 B C must have been typhus, but the account leaves some doubt despite the vivid description given by Thucydides

The word "typhus" is derived from the Greek, *typhos*, meaning smoky or hazy Although the term had been used by Hippocrates to depict a "confused state of intellect with a tendency to stupor," it was not actually applied to typhus fever until 1760 when Sauvages selected it to describe the mental

Skipper, E, Beverley, J K, Beattie, C. P, 1954, Acquired toxoplasmosis with a report of two cases simulating glandular fever and one possible case resembling typhus, Lancet *1*, 287-290

Smith, C H, 1944, Acute infectious lymphocytosis, a specific infection report of 4 cases showing its communicability, J.A.M.A *125*, 342-349

Sohier, R, Lépine, P, and Sautter, V, 1940, Recherches sur de la transmission experimentale de la mononucleose infectieuse au singe et a l'homme, Ann Inst Pasteur *65*, 50-62

Sprunt, T P, and Evans, F A, 1920, Mononuclear leucocytosis in reaction to acute infections (infectious mononucleosis), Bull Johns Hopkins Hosp *31*, 410-417

Stuart, C A, Welch, H, Cunningham, J S, and Burgess, A M, 1936, Infectious mononucleosis, Arch Int Med *58*, 512-522.

Thomsen, S, 1942, Studies Over Mononucleosis Infectiosa (with an English summary), Copenhagen, Munksgaard

Thurm, [redacted] H, and Bassen, F, 1955, Infectious mononucleosis and acute hemolytic anemia, report of 2 cases and review of literature, Blood *10*, 841-851

Tidy, H L., 1934, Glandular fever and infectious mononucleosis, Lancet *ii*, 180-186, 236-240

Tidy, H L, and Morley, E B, 1921, Glandular fever, Brit M J *1*, 452-456

Watson, J, Johnson, P, Kahn, J, and Stone, F M, 1951, Subclinical infectious mononucleosis with hepatitis, Arch. Int Med *88*, 618-626

Wechsler, H F, Rosenblum, A H, and Sills, C. T, 1946, Infectious mononucleosis; report of an epidemic in an Army post, Ann Int Med *25*, 113-133, 236-265

West, J P, 1896, An epidemic of glandular fever, Arch Pediat *13*, 889-900.

Wising, P J, 1942, Successful transmission of infectious mononucleosis to man by transfusion of heparinized blood? Acta med scandinav. *109*, 507-513

Zarafonetis, C J D, Oster, H L, and Colville, V. F, 1953, Cold agglutination of sheep erythrocytes as a factor in false-positive heterophile agglutination tests, J Lab & Clin Med *41*, 906-912

CLINICAL PICTURE

The incubation period usually is from 10 to 14 days, but it may be shorter if the infecting dose is unusually large. Prodromal symptoms occur infrequently; if present, they are headache, lassitude and weakness, sometimes accompanied by slight fever. Onset is usually abrupt, and patients are frequently able to state the exact hour at which they noted the beginning of their illness The first symptoms are malaise, chilly sensations, headache, weakness, generalized aches and pains During the first 2 or 3 days the temperature may fluctuate from normal to 39° C, but after the third day it attains a level of 39° to 41° C where it remains until death or recovery of the patient (Fig 120).

There may be one or more shaking chills early in the first week Headache increases in severity and may be generalized or most severe in the frontal region, it is one of the most constant features of typhus fever, and efforts to diminish its intensity usually fail Patients tend to cough in the first week of disease without raising much, if any, sputum Vomiting occurs infrequently with the onset of the disease and is rare after the third day, except when azotemia develops Constipation is far commoner than is diarrhea Patients may appear to be deaf, and they often complain of ringing in the ears or vertigo Pains in the muscles of the back and the legs may be very troublesome The appearance of a generalized eruption, usually between the 4th and the 7th days of the disease, is an important feature Preceding the rash in some instances, there may be a transient blotchy erythema or a marbled appearance of the skin, sometimes referred to as subcuticular mottling The characteristic rash is first apparent on the trunk, spreading in the course of 1 or 2 days over the entire body except the face, the palms and the soles, which are involved only in gravely ill patients Lesions have been recognized on the soft palate in rare instances At first the skin lesions are macules or maculopapules about 2 to 4 mm in diameter, pinkish to bright red in color, with rather indefinite borders Slight pressure causes them to fade completely This eruption has been described as a "mulberry rash" (Fig 121)

The rash may be absent throughout the

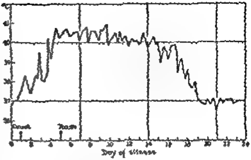

FIG 120. Temperature curve, epidemic typhus fever

disease in perhaps 10 per cent of light-skinned persons, lesions are even more difficult to detect in dark-skinned persons In the first few days, the pulse rate may not be quite so high as the temperature would warrant, but toward the end of the first week and for the remainder of the disease the pulse rate is rapid in proportion to the temperature The blood pressure is usually low, and there may be brief periods of severe hypotension The respiratory rate is quite often increased out of proportion to any findings in the chest Toward the end of the first week, patients exhibit varying degrees of photophobia and suffusion of the conjunctivae The face is often flushed and sometimes assumes a dusky appearance Some observers have commented on the presence of facial edema in certain patients Delirium may appear, but it is more common to observe only mental dullness or slight stupor at this stage The urine frequently is reduced in amount, and the specific gravity is elevated Sometimes there may be inability to void or incontinence of urine and feces, although these usually do not occur before the 2nd or 3rd week

In the 2nd week of illness, the skin lesions become darker in color, assuming a reddish to reddish-purple hue, pressure no longer causes them to fade In mild cases the rash may last for 2 or 3 days and then disappear In moderately or severely ill patients, the lesions are usually visible until the end of the febrile period In many of the severe cases the rash becomes petechial or even frankly hemorrhagic Confluence of the rash has been noted in very severely ill patients In general, there

state of patients suffering from the disease It should be emphasized that *typhus* and *typhoid* fevers were frequently confused until 1837 when W. W. Gerhard in Philadelphia called attention to the important clinical and pathologic differences between the two diseases Confusion in terminology persists even now, since *typhoid fever* is called *typhus abdominalis* in some parts of Europe.

Typhus fever always has been intimately associated with wars, famines and human misfortunes Its effect on the outcome of battles has often been decisive The earliest military chronicle implicating typhus is that which describes the siege of Granada in 1489, there were 17,000 deaths from typhus in the Spanish Army, almost [illegible] times the number killed in combat with the Moors Perhaps typhus was brought to Spain by the Spanish soldiers who had fought against the Turks in Cyprus. Soon thereafter the disease broke out in Italy where Fracastorius had occasion to study its characteristics In 1528, the French Army besieging Naples was at the point of decisive victory over the forces of Charles V, a victory which would have had enormous effects on the subsequent developments in Europe. But then " [illegible] typhus made its political debut—by one of the most far-reaching and profoundly effective strokes of its entire career [illegible]." (Zinsser, 1935) With great rapidity it struck down 30,000 soldiers in the camps of the French, and the remnants of the army were forced to withdraw

The Balkan campaigns in the 16th century contributed greatly to the spread of typhus across Europe Large forces were assembled from various parts of Germany, Italy and France for combat with the Turks, but many of the men were stricken by typhus before they reached the battlefields The disease became known as the "morbus hungaricus," since it was disseminated throughout Europe by [illegible] first [illegible] Nev[illegible] Sab[illegible] refers to the severe epidemic in the highlands of Mexico in 1576 and 1577 during which more than 2 million Indians died. Although the records suggest that typhus fever may have existed in the New World before the arrival of the Spanish explorers, it is not possible to decide this point on present evidence.

The disaster which befell Napoleon's army of half a million men in 1812 was in part the work of typhus. During the period from 1816 to 1819, a great epidemic of typhus is said to have caused at least 700,000 cases among the 6 million inhabitants of Ireland Typhus tended to subside somewhat in the last half of the 19th century, but it reappeared again in World War I, striking Serbia severely in 1915. There were only 400 Serbian doctors, and almost all of them contracted typhus; 126 of these doctors died. The mortality in the civilian population ranged from approximately 20 per cent during the rise and decline of the epidemic, to 60 and even 70 per cent at its height. In less than 6 months more than 150,000 Serbians died of typhus (Strong et al, 1920). Between 1918 and 1922, typhus ravaged Russia; estimates place the number of cases as high as 30 million in this period and deaths as many as 3 million

During World War II typhus more than once threatened to complicate military operations The areas of North Africa in which [illegible] in Naples just as that badly overcrowded and heavily bombed city was occupied by the Allied forces. In 1944, the Yugoslavian army, engaged in bitter struggle with the Germans, was severely handicapped by the spread of typhus not only in the civilian population but in the army itself. As the Allied armies crossed Germany in 1945, typhus was encountered in many of the notorious Nazi concentration camps. Although only a very short interval elapsed between the liberation of these camps and the arrival of adequate forces to maintain discipline and control from the sanitary point of view, many of the louse-infested, typhus-infected inmates escaped into the surrounding countryside, scattering far and wide. Attempts to restrict the spread of typhus were vastly handicapped by the floods of persons who had been transplanted into Germany from occupied countries; more than a million of these "slave laborers" were freed by the Allied armies, and nearly all crowded the roads seeking to return to their own countries. Shortly after the cessation of hostilities in the Far East, Japan and Korea underwent a severe typhus epidemic of approximately 26,000 cases.

The examples cited above suffice to indicate that epidemic typhus has been one of the major epidemic diseases in the history of man It is probable that typhus fever has been exceeded only by malaria as a cause of widespread human suffering.

acter increases The parts subjected to pressure and particularly the skin over the sacrum become red and tender, and are liable to slough The pulse ■ frequent (112 to 140), small, weak, and undulating, and not unfrequently [sic] intermittent, irregular, or scarcely perceptible, the cardiac impulse and systolic sound of the heart are diminished in intensity, or absent (Murchison, 1884, pp. 122-123)

To this account a few more details may be added to complete the picture of the critical period of the illness Persistent cough with difficulty in expectoration may be accompanied by development of a patchy pulmonary consolidation, more often disclosed by roentgenograms than by physical examination *Respirations are usually rapid and somewhat shallow*. The blood pressure continues to be lower than normal and may fall below 80/50 in severe cases. *Although the myocardium is damaged, the syndrome of congestive heart failure does not appear in the acute stage of the disease Gallop rhythm often develops* in severely ill patients The spleen becomes palpable in about half the cases. Renal insufficiency of varying degree is a common occurrence Oliguria and elevated blood urea nitrogen *are nearly always features of the* clinical course of fatal cases of typhus fever. (Fig 122) Gangrene of the toes, feet, tips of fingers, *ear lobes, nose, penis, scrotum* or vulva may occur Parotitis, otitis media and furunculosis are common complications which may appear toward the end of the 2nd week of illness

In fatal cases, the terminal period is usually characterized by profound stupor which

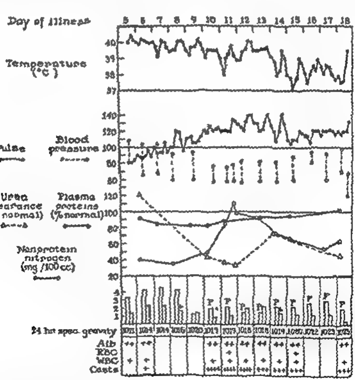

FIG 122 Graphic representation of clinical findings on a male patient, 46 years old, admitted on 5th day of disease, dying on 18th day In space on chart directly above "specific gravity," data about fluid intake and output (liters/24 hrs) are portrayed, P represents fluid given parenterally (Yeomans, A, Snyder, J C, Murray, E S, Ecke, R S, and Zarafonetis, C J D, 1945, Azotemia in typhus fever, Ann Int Med 23, 735)

FIG 121 Typical rash of typhus fever, 11th day of disease (U S A Typhus Commission)

is no residual evidence of the rash after recovery; in rare instances, however, brownish areas of pigmentation are visible for several months If a patient dies, the rash ordinarily persists after death, particularly in dependent areas.

The 2nd and 3rd weeks of illness constitute the critical period. Increasing prostration develops, and patients are unable to eat or drink without assistance. Mental dullness supervenes, and patients seem to be quite deaf. The dullness may progress to stupor or coma. The stupor may be interrupted by brief episodes of delirium in which patients become active or even violent and then lapse back into apathy. They may mutter to themselves or carry on conversations with imaginary friends. In rare instances, stiffness of the neck may develop in sufficient degree to prompt the performance of a lumbar puncture; the spinal fluid may be under slightly increased pressure but otherwise is normal Murchison gave the following account of the clinical picture of typhus in the 2nd week of the disease which is remarkable for its accuracy:

. . . the stupor and delirium alternate, the latter being most marked in the night-time. The prostration is extreme the patient lies on his back, moaning, muttering incoherently, or still and motionless, with a tendency to sink to the bottom of the bed. He is quite unable to raise himself, or even to turn on his side, is with difficulty aroused, and is utterly indifferent to surrounding objects and persons Tremors, subsultus, and picking of the bedclothes may be observed The expression is stupid and vacant; the conjunctivae are injected, the eyelids for the most part closed, and the pupils often contracted. Deafness is not uncommon. If spoken to loudly, the patient opens his eyes and stares vacantly at those about him, and when told to put

mind is far from inactive, the imagination conjures up the most frightful fancies, to which implicit belief is attached, and of which a distinct recollection may remain after recovery The teeth and lips are now covered with sordes; the tongue is hard and dry, dark brown or black, contracted into a ball, tremulous and protruded with difficulty or not at all The abdomen is flaccid, or sometimes tympanitic; the bowels are still confined, or one or two slightly relaxed motions are passed daily in bed The urine is more copious, but paler, and of low specific gravity, and is

after initiation of intensive therapy, the progress of the disease is usually arrested, and improvement occurs within 2 to 3 days, as the temperature subsides and the prostration and the mental apathy lessen The earlier that treatment is begun in the illness, the more prompt the recovery and the shorter the period of convalescence Treatment may fail if not administered before the patient has developed severe azotemia and extensive tissue damage from vascular lesions

Pathologic Picture

There are no gross findings characteristic of typhus fever at necropsy except the skin lesions Bronchopneumonia, myocardial changes and petechial hemorrhages in the subcutaneous tissues and the brain are the principal features which may be observed Rarely, symmetrical gangrene of the extremities and thrombosis of a large blood vessel may be present The microscopic pathology of typhus is quite characteristic Rickettsiae multiply inside endothelial cells lining small blood vessels Affected cells become swollen, and proliferation occurs as shown by numerous mitotic figures Thrombosis results from injury caused by rickettsial growth Accumulation of polymorphonuclear leukocytes, macrophages and lymphoid cells around such lesions in capillaries, arterioles or venules gives rise to distinctive histologic appearance sometimes referred to as Fraenkel's nodules (Fig 123) Early stages in the development of these lesions have been studied by skin biopsies (Wolbach, Todd and Palfrey, 1922) Rickettsiae can be demonstrated by careful technic in some of the endothelial cells Vascular lesions are most numerous in the skin,

Fig 123 Section of brain of patient who died of typhus fever on 13th day of disease, showing a typical typhus nodule in the medulla oblongata, note proliferative character of the lesion (×275) (Dr W B MacAllister and the U S A Typhus Commission, photograph by Dr. R M Allen)

changes to coma; some patients have other features characteristic of uremia. Cyanosis deepens and pulmonary consolidation develops to an extent readily detectable by physical examination The blood pressure may fall to a very low level Evidence of peripheral vascular collapse may be noted shortly before death. The skin becomes cold and moist with a livid appearance; the pulse becomes very faint or absent at the wrist, and death ensues. In some instances, the temperature may drop to subnormal values a few hours before death Unless the fatal termination is due to secondary bacterial infection, death from typhus per se usually occurs between the 9th and the 18th days of illness.

If a patient recovers, the fever generally subsides by rapid lysis in the 3rd week of illness, the temperature becoming normal or subnormal within 2 or 5 days. The mental state improves strikingly when the temperature begins its descent With the exception of infrequent cases of very severe encephalitis due to typhus itself, recovery of normal mental and physical capacities is remarkably rapid in convalescence Full strength and activity are regained in 2 or 3 months. An astonishing feature of the disease is the absence of serious sequelae, despite the fact that the central nervous system, the myocardium and the kidneys are dangerously involved in the acute stage.

The hemoglobin and red blood cell count decrease during the course of typhus, particularly in the 2nd and the 3rd weeks of illness when the red count may fall to 3 5 million cells per cu mm with a corresponding reduction in hemoglobin values. Return to normal levels usually is rapid In the first week of illness, the white blood cell count tends to be somewhat reduced, values range from 8,000 to 2,000 cells per cu. mm. Differential counts are interesting chiefly because of the constant absence of eosinophils during the febrile period In the 2nd and the 3rd weeks of illness, the white blood cell count may be normal or slightly elevated. In the presence of secondary bacterial complications, the leukocyte count may be considerably above normal. Practically all patients have albumin in the urine in varying amounts while fever is present. There is a tendency for the specific gravity of the urine to be high during the 1st week, possibly because of dehydration. In the 2nd and the 3rd weeks of the disease, severely ill patients may excrete only small amounts of urine of relatively low specific gravity. Diuresis has been observed at the beginning of the convalescent period in some patients. Red blood cells may occur in the urine in varying numbers, but gross hematuria is a very rare finding. However, granular casts may be numerous, particularly when patients have nitrogen retention. The nonprotein nitrogen and the urea nitrogen of the blood are increased in about half the cases The rise begins in the 2nd week of illness and often follows a drop of blood pressure, although not invariably. Values between 100 and 200 mg. per 100 ml. for the blood nonprotein nitrogen have been observed with survival of the patient, but usually such levels presage fatal outcome A reduction in renal plasma flow may be responsible for the kidney damage The appearance of renal insufficiency as indicated by azotemia is one of the earliest laboratory findings of serious prognostic import; azotemia precedes death almost without exception. Changes in serum proteins are observed toward the end of the first week of illness; serum albumin is reduced, and serum globulin is increased, leading to a reversal of the albumin-globulin ratio Serum chlorides are usually reduced below 95 milliequivalents per liter, and values as low as 85 may be encountered; the chlorides in the urine are concomitantly reduced Carbon dioxide combining power of the blood is either normal or reduced, serum pH is normal in most instances. Likewise, values for plasma volume are within the accepted normal range The slight reduction in whole blood volume encountered toward the end of the disease in some patients is attributed to a reduction in red cell volume alone Roentgenograms of the chest frequently reveal a diffuse mottling of various areas in the lung field, often more extensive than the physical signs of pulmonary involvement would indicate. Transient abnormalities in the electrocardiogram have been recorded, these consist of low voltage of the QRS complexes, low or inverted T waves and, less often, depression of the S-T segment.

The clinical course of typhus is profoundly altered by treatment with the tetracyclines or chloramphenicol Within 12 to 24 hours

tion by developing a fever of several days' duration Infection is established in guinea pigs by the intra-abdominal inoculation of blood taken from a typhus patient during the febrile period. If the specimen is obtained in the first week of illness, whole blood may be used, after the 7th or 8th day of illness it is advisable to allow the specimen to clot, after centrifugation the serum is separated and stored for use in serologic tests, then the clot is ground with an equal volume of a sterile diluent, such as skimmed milk or the diluent referred to as "sucrose—PG," which is a solution containing sucrose, phosphate and glutamate (Bovarnick et al, 1950) After allowing gross particles to settle out, the suspension of ground clot is inoculated into 2 male guinea pigs, each weighing about 500 Gm.; each animal is given 4 to 5 ml Female pigs may be employed successfully, but, for reasons to be mentioned in the section on murine typhus, male guinea pigs are preferred Removal of the serum from the clot serves to increase the chance of successful detection of rickettsiae by eliminating antibodies which are present in the patient's blood serum after the 7th day of illness In some instances the pigs may be sick during the first 18 to 24 hours after the inoculation, probably as a consequence of the large volume of blood which is required for successful isolation of rickettsiae A small percentage of animals may succumb at this stage Usually, however, the animals remain entirely well after the inoculation, and the detection of the rickettsial infection depends solely on the temperature curve The normal morning temperature of a guinea pig varies between 38° and 39.5° C, values above 40.0° C are indicative of fever Ordinarily, guinea pigs which are inoculated with a patient's blood have a somewhat prolonged incubation period before their temperature exceeds 40.0° C, 12 to 24 days may elapse before the rise occurs When suspensions of brain or spleen of such animals are removed on the 3rd or 4th day of fever for subinoculation to fresh guinea pigs by the intra-abdominal route, the incubation period is shortened to 7 to 9 days and remains at this interval in successive transfers made in this manner

To accomplish a transfer, a febrile guinea pig is lightly anesthetized with ether and bled out from the heart, the brain is removed and ground to a 10 per cent suspension in a suitable diluent One or 2 ml. of the suspension is used for the intra-abdominal inoculation of each guinea pig in the next passage Spleen may be employed successfully instead of brain if taken on the 2nd or 3rd day of fever. The only evidence of infection with *R. prowazeki* to be observed grossly at the time of sacrifice of the guinea pig is a fibrinous exudate over the surface of the spleen; this is a constant finding on first passage as well as in subsequent transfers Direct smears made by scraping a few cells from the surface of the spleen just beneath this exudate, when stained appropriately, may contain a few large serosal cells in which rickettsiae are found It is extremely difficult to find cells containing rickettsiae in smears of spleen or tunica vaginalis in guinea pigs infected with epidemic strains unless the search is made on the 1st or 2nd day of the febrile reaction The fever may last for only 2 or 3 days, more commonly about a week, rarely longer than 10 days Guinea pigs survive typhus infection without sequelae unless the infecting dose is a massive one e g, large volume of infected yolk sac containing billions of rickettsiae per ml, in such circumstances the incubation period lasts only a few hours, and, in addition to fever, the animal may show enlargement of the scrotum and adhesions between the testes and the scrotal sac This "tunica reaction" or "scrotal swelling" was first observed by Neill in 1917 and further studied by Mooser in 1928, the phenomenon is sometimes called the Neill-Mooser reaction Although it may be observed in pigs infected with epidemic typhus, particularly if numerous rickettsiae are present in the inoculum, the reaction is more common in murine typhus

Guinea pigs do not develop a skin eruption as a consequence of typhus infection, but microscopic examination of the brain taken in the period from the 3rd or 4th day of fever up to a few days after the end of the fever shows the presence of many vascular lesions similar to those found in the brains of man and monkeys A serious disadvantage to the use of guinea pigs for the laboratory study of epidemic typhus is the fact that the only evidence of infection is obtained by taking the rectal temperatures daily for several

the central nervous system and the myocardium but are scattered widely throughout different organs of the body. Necrotic areas of the skin appear to be associated with thrombosis of capillaries, small arteries and veins, beginning in the corium Symmetrical gangrene of the extremities may be due to nerve lesions instead of thrombosis of large vessels. Lesions in the respiratory tract are similar to those in terminal bronchopneumonic processes in various diseases. Suggestive evidence of a rickettsial pneumonia has been reported (National Research Council, 1953).

Experimental Infection; Host Range

Epidemic typhus is a disease which occurs as a natural infection only of man, the human body louse, *Pediculus humanus corporis*, and the human head louse, *Pediculus humanus capitis*. The role of the human body louse in the transmission of typhus was first demonstrated experimentally by Nicolle, Comte and Conseil in 1909. The human body louse spends its entire existence in the clothes of man Eggs are laid in the seams of the undergarments After about 8 days, the eggs hatch, and the nymphs in the course of 2 weeks go through 3 moults to become adults The insects crawl about on the clothes, leaving the garments only to take a blood meal from their host. Lice cannot fly or jump but they have been observed to crawl for several yards. Each louse takes 4 to 6 blood meals a day from its host under natural conditions Human blood constitutes their only food Rickettsiae are present in the blood of patients suffering from typhus during the febrile period of the disease The body louse becomes infected by imbibing a blood meal containing rickettsiae, which then enter cells lining the intestinal tract of the louse. All stages of lice, whether newly hatched nymphs or fully developed adults, are susceptible to infection with *R. prowazeki*. After a few days the rickettsiae have multiplied so profusely that the cells containing them are swollen and may burst The organisms may then be passed in the feces of the louse or may enter uninvolved cells lining the intestinal tract. Ordinarily, rickettsiae appear in the feces of a typhus-infected louse about 3 to 5 days after the first infective meal. The louse usually succumbs to the infection after 7 to 10 days, but it is important to note that 24 days may elapse before all cells of the mid-intestine become full of rickettsiae.

Lice have been used extensively in typhus research. For this purpose a colony of normal stock lice is maintained by feeding twice daily on healthy human subjects The lice are confined in a small capsule covered with bolting silk which is strapped to the leg or the arm. Sometimes it is more convenient to store the capsule in an incubator between feedings. If the temperature of the incubator is lower than 30° C or higher than 37° C, rickettsiae may fail to develop; at temperatures above 37° C. the louse colony itself fares poorly. An ingenious technic was developed by Weigl in 1920 for the experimental infection of lice by means of a glass capillary inserted into the insect's rectum. Lice thus infected develop typhus which is similar in every respect to the infection acquired by feeding Although lice ordinarily do not thrive if they feed on other species than man, it has been possible to nourish them indefinitely on rabbits or to infect them by giving the rabbits a large inoculum of rickettsiae intravenously. More recently an artificial membrane has been developed for the experimental infection of lice (Haddon, 1956a, b), thereby permitting a wider range of experiments with these insects

Rickettsiae prowazeki are present only in the intestinal lining cells and the feces of infected lice; they have not been demonstrated in other tissues, such as the salivary glands They are not passed from generation to generation of lice in the egg. The course of typhus infection in the human head louse is identical with that in the body louse, but the latter is far more important in transmission of epidemic typhus

Monkeys, guinea pigs, rats and other rodents, developing chick embryos and certain arthropods are susceptible to experimental infection with epidemic typhus. Monkeys inoculated with *R. prowazeki* undergo a febrile illness of a few days' duration from which they survive. A skin eruption has been described in typhus-infected monkeys, but usually this is absent. The animals suffer loss of appetite and become apathetic, but otherwise exhibit no evidence of illness

Typhus was transmitted to guinea pigs by Nicolle, Conseil and Conor in 1911, who observed that the animals responded to inocula-

tion by developing a fever of several days' duration. Infection is established in guinea pigs by the intra-abdominal inoculation of blood taken from a typhus patient during the febrile period. If the specimen is obtained in the first week of illness, whole blood may be used; after the 7th or 8th day of illness it is advisable to allow the specimen to clot; after centrifugation the serum is separated and stored for use in serologic tests, then the clot is ground with an equal volume of a sterile diluent, such as skimmed milk or the diluent referred to as "sucrose—PG," which is a solution containing sucrose, phosphate and glutamate (Bovarnick et al, 1950). After allowing gross particles to settle out, the suspension of ground clot is inoculated into 2 male guinea pigs, each weighing about 500 Gm, each animal is given 4 to 5 ml. Female pigs may be employed successfully, but, for reasons to be mentioned in the section on murine typhus, male guinea pigs are preferred. Removal of the serum from the clot serves to increase the chance of successful detection of rickettsiae by eliminating antibodies which are present in the patient's blood serum after the 7th day of illness. In some instances the pigs may be sick during the first 18 to 24 hours after the inoculation, probably as a consequence of the large volume of blood which is required for successful isolation of rickettsiae. A small percentage of animals may succumb at this stage. Usually, however, the animals remain entirely well after the inoculation, and the detection of the rickettsial infection depends solely on the temperature curve. The normal morning temperature of a guinea pig varies between 38° and 39.5° C, values above 40.0° C are indicative of fever. Ordinarily, guinea pigs which are inoculated with a patient's blood have a somewhat prolonged incubation period before their temperature exceeds 40.0° C, 12 to 24 days may elapse before the rise occurs. When suspensions of brain or spleen of such animals are removed on the 3rd or 4th day of fever for subinoculation to fresh guinea pigs by the intra-abdominal route, the incubation period is shortened to 7 to 9 days and remains at this interval in successive transfers made in this manner.

To accomplish a transfer, a febrile guinea pig is lightly anesthetized with ether and bled out from the heart; the brain is removed and ground to a 10 per cent suspension in a suitable diluent. One or 2 ml. of the suspension is used for the intra-abdominal inoculation of each guinea pig in the next passage. Spleen may be employed successfully instead of brain if taken on the 2nd or 3rd day of fever. The only evidence of infection with *R. prowazeki* to be observed grossly at the time of sacrifice of the guinea pig is a fibrinous exudate over the surface of the spleen; this is a constant finding on first passage as well as in subsequent transfers. Direct smears made by scraping a few cells from the surface of the spleen just beneath this exudate, when stained appropriately, may contain a few large serosal cells in which rickettsiae are found. It is extremely difficult to find cells containing rickettsiae in smears of spleen or tunica vaginalis in guinea pigs infected with epidemic strains unless the search is made on the 1st or 2nd day of the febrile reaction. The fever may last for only 2 or 3 days, more commonly about a week, rarely longer than 10 days. Guinea pigs survive typhus infection without sequelae unless the infecting dose is a massive one, e.g., large volume of infected yolk sac containing billions of rickettsiae per ml., in such circumstances the incubation period lasts only a few hours, and, in addition to fever, the animal may show enlargement of the scrotum and adhesions between the testes and the scrotal sac. This "tunica reaction" or "scrotal swelling" was first observed by Neill in 1917 and further studied by Mooser in 1928, the phenomenon is sometimes called the Neill-Mooser reaction. Although it may be observed in pigs infected with epidemic typhus, particularly if numerous rickettsiae are present in the inoculum, the reaction is more common in murine typhus.

Guinea pigs do not develop a skin eruption as a consequence of typhus infection, but microscopic examination of the brain taken in the period from the 3rd or 4th day of fever up to a few days after the end of the fever shows the presence of many vascular lesions similar to those found in the brains of man and monkeys. A serious disadvantage to the use of guinea pigs for the laboratory study of epidemic typhus is the fact that the only evidence of infection is obtained by taking the rectal temperatures daily for several

weeks. Approximately 5 per cent of guinea pigs inoculated with fully virulent material fail to exhibit a febrile response.

The cotton rat (*Sigmodon hispidus*) has been very useful in quantitative studies with typhus rickettsiae. This species succumbs to infection following intracardial inoculation of 10^6 to 10^7 living typhus rickettsiae It develops a definite immunity by 21 days after intra-abdominal inoculation of very small numbers of rickettsiae; complement-fixation tests become strongly positive, and the animals then resist several fatal challenge doses The minimal number of typhus rickettsiae detectable in cotton rats is approximately the same as that required for detection in human body lice (Fuller, 1953, 1954). By contrast, several times as many are usually required for detection in guinea pigs

The white rat is not susceptible to infection with epidemic typhus in the usual sense, it undergoes only an inapparent infection An attempt to maintain a strain by serial passage in white rats usually is unsuccessful, a phenomenon of value in differentiating murine from epidemic typhus

The white mouse undergoes only an inapparent infection when inoculated by the intra-abdominal route with most strains of epidemic typhus if the inoculum contains relatively few *R prowazeki* However, it is possible to maintain a strain for several passages in mice by intra-abdominal inoculation of brain suspensions. Intranasal inoculation of rich suspensions of *R prowazeki* is followed by pulmonary consolidation and death; strains have been maintained in mice indefinitely by passage in this manner. Exposure of white mice to large doses of x-ray increases their susceptibility, and small numbers of rickettsiae given by the intra-abdominal route then produce a fatal infection within several days. Intravenous or intra-abdominal inoculation of concentrated suspensions of living, fully virulent rickettsiae into normal white mice is followed in a few hours by death of the animals as a consequence of a toxic reaction. The white rat also succumbs to the toxic property of epidemic typhus rickettsiae a few hours following intravenous inoculation of concentrated suspensions of the micro-organisms This phenomenon is discussed in the section on etiology.

Various other species have been employed in laboratory experiments with typhus, including the South African gerbil, *Tatera* [illegible] r- [illegible] s-pects of typhus studies. Under certain conditions a rich suspension of rickettsiae has been obtained from rabbit lungs following intratracheal inoculation. Giroud in 1938 showed that lesions in the skin of rabbits could be produced by intracutaneous inoculation of rickettsiae and that the technic provided possibilities for quantitative studies.

It should be emphasized that the attempt to recover typhus rickettsiae from a patient is a research problem not to be undertaken for ordinary diagnostic purposes because of the potential hazard to laboratory personnel

Etiology

The initial publications of Ricketts and Wilder (1910a, b) and von Prowazek (1914) were followed, after a period of some uncertainty as to the specific etiologic agent, by da Rocha Lima's studies (1916) which clearly implicated the micro-organisms which he named. The carefully controlled experiments of Wolbach et al. (1922) eliminated all doubts as to the causative relationship of *Rickettsia prowazeki* to epidemic typhus

There is remarkable variation in the size and the shape of typhus rickettsiae (Fig. 124) In smear preparations, they appear as minute coccoid or rod-shaped organisms, frequently occurring in pairs, sometimes in long chains, having a diameter of approximately 0 3 μ The organisms often have a bipolar appearance in stained preparations and when visualized by phase contrast microscopy (Ris and Fox, 1949). It is difficult to stain *R. prowazeki* by Gram's method. The method described by Macchiavello (1937) is the most satisfactory stain for rickettsiae.

Directions for Macchiavello's stain: Solutions· (A) 0.25 per cent basic fuchsin in distilled water; (B) *freshly prepared 0 25 to* 0.5 per cent citric acid; (C) 1 per cent methylene blue in distilled water. The slide is fixed lightly in heat, stained for 3 to 5 minutes with freshly filtered fuchsin; the fuchsin is poured off the slide which is then quickly dipped in the freshly prepared citric acid so-

lution, it is removed immediately and placed in a dish containing running tap water The final step is the flooding of the slide with methylene blue which is poured off after a few seconds The slide is then washed briefly in running tap water and dried with a piece of filter paper. Rickettsiae are stained a bright pink or red against a bluish background

R. prowazeki is relatively labile and easily killed by the common antiseptics such as formalin, phenol, merthiolate, etc Temperatures above 56° C. for 30 minutes result in its death. Viability may be retained in blood specimens at icebox temperature (+2° to +4° C.) for one or more days, but the organisms die in a few hours at room temperature or at 37° C. *R. prowazeki* in louse feces remains viable for several months if the temperature and the humidity are low Tap water, distilled water and ordinary physiologic saline have a deleterious effect on its viability, sterile skimmed milk or "sucrose PG" (Bovarnick, Miller and Snyder, 1950) is the most satisfactory medium for preserving viability of rickettsiae in suspensions The most satisfactory method of preserving rickettsiae is quick freezing in an alcohol-dry-ice mixture in a sealed glass ampule with subsequent storage in a dry-ice cabinet (−76° C.); in this state rickettsiae retain their viability for at least 16 years Rapid thawing is important, however, since slow return from the temperature of −76° C to room temperature results in great loss of viability Furthermore, even brief periods of storage at temperatures between −5° and −20° C may result in loss of viability Desiccation from the frozen state is satisfactory for storage of strains under appropriate circumstances By various technics evidence has been obtained which indi-

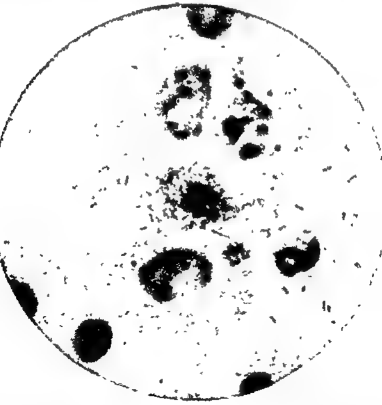

Fig 124 Microphotograph showing typhus rickettsiae inside and outside of cells Macchiavello's stain (×2000) (Photograph by Dr R M Allen)

cates that typhus rickettsiae contain carbohydrate-protein complexes, nucleic acids and lipids (Castaneda, 1934; Cohen and Chargaff, 1944; Cohen, 1950, Ris and Fox, 1949; Schaechter et al, 1957).

When care is taken to preserve the viability of typhus rickettsiae during purification procedures, it is possible to demonstrate that these micro-organisms have independent respiratory activity (Bovarnick and Snyder, 1949; Wisseman et al, 1951); the presence of a transaminase in rickettsiae has also been demonstrated (Bovarnick and Miller, 1950; Hopps et al, 1956)

Many attempts have been made, without success, to cultivate *R prowazeki* in cell-free media Cultivation in various types of tissue culture has been accomplished. Castaneda in 1939 showed that an abundance of rickettsiae could be obtained by the intranasal inoculation of rodents with murine typhus, his observations were extended to the epidemic strains by French workers (Durand and Giroud, 1940) The yolk-sac membrane of developing chick embryos was shown by Cox (1938, 1941, 1948) to be an excellent medium for the cultivation of *R. prowazeki* and several other species of rickettsiae In general, this technic is the most widely used at the present time Material containing rickettsiae is inoculated directly into the yolk sac of a 6- or 7-day-old embryonated hen's egg After several days' incubation at 34° to 36° C, the yolk-sac membrane is removed for examination or for subsequent inoculation into other chick embryos Such infected yolk-sac membranes may contain more than 10^9 viable rickettsiae per ml Successful isolation of *R. prowazeki* from the blood or the bone marrow of patients has been accomplished by direct inoculation of blood or ground clot into developing chick embryos Rickettsiae may be few or absent in stained smears of the yolk-sac preparation in the first few transfers but become very abundant in subsequent passages. An important characteristic of *R. prowazeki* is its location only in the cytoplasm of cells, never within nuclei This feature distinguishes the typhus group from the spotted fever group, members of which do invade nuclei

Infection with *R. prowazeki* evokes serologic responses in man and lower animals; specific antibodies can be demonstrated by fixation of complement, agglutination of rickettsiae, precipitin reactions, opsonic tests, and neutralization or protection tests. There is also a very important serologic test called the Weil-Felix reaction, the agglutination of Proteus OX 19 by sera of typhus patients Weil and Felix (1916) obtained from the urine of a typhus patient a proteus strain which was agglutinated not only by the serum of the original patient but also by the sera of other typhus patients Further study of various strains of proteus revealed that the one now called OX 19 is the most suitable for diagnosis of typhus. This phenomenon has been the subject of much experimentation, and numerous hypotheses have been advanced in its explanation Although no etiologic relationship has been detected between strains of this bacterium and typhus fever, Castaneda (1934) demonstrated a carbohydrate antigen common to Proteus OX 19 and to *R. prowazeki*. Although the Weil-Felix reaction occurs in more than 90 per cent of patients suffering from proved louse-borne typhus, the sera of typhus-infected guinea pigs and some other animals fail to develop agglutinins for Proteus OX 19.

Studies by Craigie and associates (1946), Fulton and Begg (1946), and Topping et al. (1945), demonstrated the existence of two components in *R. prowazeki*, a heat-labile component which is specific for *R. prowazeki* and a heat-stable component which is common to both *R prowazeki* and *R. mooseri* By repeated washing in a high-speed centrifuge (Plotz et al, 1948), the common antigen can be reduced to a low concentration, resulting in a considerable increase in specificity of some rickettsial suspensions

Living typhus rickettsiae, both *R. prowazeki*, and *R. mooseri*, are toxic for certain species if administered in highly concentrated suspensions (Gildemeister and Haagen, 1940, Neva and Snyder, 1952, 1955; Wattenburg et al, 1955). The toxicity is intimately related to living rickettsiae; the mode of action is believed to be a direct injury to the cells of the animal, usually the capillary endothelium with resultant leakage of plasma and death from progressive loss of circulating blood volume In rabbits, suspensions of *R. mooseri* cause lysis in vivo of red blood

cells and a rise in blood potassium which contributes to the manifestations of severe toxicity (Paterson et al, 1954)

The red blood cells of several species are lysed in vitro by typhus rickettsiae (Clarke and Fox, 1948, Snyder et al, 1954) It is probable that the phenomenon depends upon at least one and probably several enzymatic reactions, glutamate and magnesium are required for the reaction

Both the toxicity and the hemolysis can be neutralized by sera of man and lower animals convalescent from typhus Further studies by Topping and associates (1945), Hamilton (1945), and Craigie and associates (1946) indicate that the toxic factors of murine and epidemic rickettsiae are immunologically distinct

Diagnosis

Before the appearance of the characteristic rash, and on clinical grounds alone, it is impossible to assert with accuracy that a patient is suffering from typhus The clinical picture of the early stage of several acute infectious diseases closely resembles that of epidemic typhus Those which are likely to be confused with it are murine typhus, smallpox, relapsing fever, malaria, typhoid fever, meningococcic meningitis, measles and yellow fever The appearance and the evolution of the typhus rash serve to distinguish it from eruptions which are features of certain other acute infectious diseases In differentiating typhus from Rocky Mountain spotted fever it is helpful to recall that the rash in the latter disease ordinarily appears first on the exposed extremities and then extends to the trunk, frequently involving the face, the palms, and the soles as well The clinical diagnosis of epidemic typhus is particularly difficult in children or in persons who have previously received antityphus vaccine In such instances, the rash may be of very short duration or absent, the symptoms much less severe, and the duration of the fever as short as 3 to 5 days

Agglutinins for Proteus OX 19 appear in the sera of most typhus patients during the 2nd week of illness In a high proportion of cases, the titer rises to 1/160 or higher, often attaining values greater than 1/1,000 at the peak of the response which usually occurs in the 3rd week of illness or in the first 2 weeks of convalescence The Weil-Felix titer subsides to levels below 1/160 a few weeks after the end of the disease In exceptional cases, repeated examination of a patient's serum throughout the disease and early convalescence may reveal no rise in Weil-Felix titer, or the patient may succumb without a rise in titer being observed at any time An infection caused by *Proteus vulgaris* gives rise to agglutinins against Proteus OX 19 In rare instances, a person may be encountered whose serum agglutinates Proteus OX 19, although he is not currently suffering from typhus fever, if this is the case, the titer remains static, not exhibiting the rise and fall which is characteristic of the response to epidemic typhus A rise in titer from a low level (0 or 1/20) to more than double the original value may be of diagnostic significance, but it is more reliable to require values greater than 1/160 for diagnostic significance. The test can be performed with living or killed Proteus X 19 organisms, but care must be taken to use cultures which are in the "O" form Slide Weil-Felix tests have been developed which can be performed in 3 to 5 minutes at the bedside Different strains of proteus are used for diagnosis of other rickettsial diseases, principally OX 2 and OX K The sera of typhus patients may show a slight rise in agglutinins for OX 2 but very rarely any for OX K

Cox's development (1938) of the yolk-sac technic for the cultivation of rickettsiae made it possible to prepare large amounts of antigen for serologic tests Suspensions of rickettsiae derived from yolk sac after repeated washing yield specific antigens suitable for differentiation of antibodies of epidemic typhus from those of murine typhus in the sera of man and lower animals Complement-fixing antibodies may be detected in the sera of patients as early as the 7th or the 8th day of illness, they increase in titer, reaching a peak by the 12th to the 16th day after onset Subsequently, the titer usually falls slowly over a period of months to low values which may persist for years Occasionally, titers may fall to zero a few weeks after the end of the disease It may be difficult or impossible to differentiate epidemic typhus from murine typhus by complement-fixation tests

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trol of active delirium or restlessness and are preferable to morphine for this purpose Barbiturates should be avoided, since some typhus patients appear to react unfavorably to them. Codeine may be required for the relief of headache. Digitalis and other drugs which act principally on the heart are rarely indicated Oxygen by face mask or tent appears to make patients more comfortable when cyanosis is present, but it is doubtful whether oxygen administration has done more than prolong life for a few hours in cases with deep cyanosis The typhus-immune sera of man and several animal species have been administered to typhus patients (cf Snyder, 1948). A beneficial effect may be observed if potent immune serum is administered very early in the disease, but such sera are expensive and not generally available

The tetracyclines and chloramphenicol are highly effective in the treatment of experimental typhus infections (Ley and Smadel, 1954). Although relatively few patients with primary epidemic louse-borne typhus have been treated with these antibiotics, their efficacy in patients with scrub typhus and Rocky Mountain spotted fever gives assurance that they will be effective against epidemic typhus as well A clinician must choose the one that he prefers on the basis of his own experience These antibiotics are usually given by mouth, although preparations suitable for intravenous use are available The initial or loading dose is given in the course of 2 to 4 hours and depends on the body weight of the patient, from 2 to 3 Gm being used for a subject weighing 70 Kg The continuing dose thereafter is 1 to 2 Gm per 24-hour period, divided into 4 doses Response to the antibiotics is observed within 24 to 48 hours Progress of the clinical course is usually arrested at whatever stage the illness had attained when treatment was begun The temperature returns to normal, and concomitantly there is rapid subjective improvement in a patient's condition. Regardless of which antibiotic is used, it should be administered for 3 days after the temperature becomes normal, to reduce the incidence of recurrent bouts of fever which may occur if therapy is withdrawn prematurely.

Penicillin and streptomycin have a slight effect on experimental rickettsial infections, but their use in man is not indicated. Sulfonamides may have a deleterious effect on typhus and are contraindicated (Snyder, 1948) If the tetracyclines or chloramphenicol are not available, the use of para-aminobenzoic acid may have a beneficial influence if administered early in the disease in large doses (cf. Snyder et al, 1947)

Epidemiology

Epidemic typhus fever has occurred in cold climates over most of the world Epidemics ordinarily reach their peak in late winter and taper off in the spring Typhus thrives under conditions of human misery which predispose to an increase in louse infestation, such as crowding of people, lack of fuel, inadequate facilities for bathing, and weather so cold that the same garments are worn continuously day and night for months at a time Persons of all ages are susceptible to typhus In children under 15 years of age, the disease is mild, probably it occurs much more frequently in young children than has been reported As age increases, the case-fatality rate rises sharply, as shown in Tables 32 and 33 In certain epidemics the case-fatality rate has been greater in males than in females in the age groups from 20 to 50 (Ecke et al, 1945) There is considerable variation in the severity of typhus in different epidemics Over-all case-fatality rates for various epidemics range from less than 10 to more than 40 per cent Very little is known of the relative resistance of different races of people to the disease, and the effect of nutrition on resistance to the disease is not well understood An attack of epidemic typhus confers immunity which persists for many years Second attacks are discussed in the section on Brill-Zinsser disease A person who has recovered from epidemic typhus is immune to murine typhus, and vice versa

R prowazeki occurs in the blood of patients during the febrile period, the human body louse becomes infected by sucking blood from them Lice tend to leave a febrile patient in favor of persons with normal temperature They quickly abandon a corpse and seek a new host Whenever a louse bites, it makes a small puncture in the skin and defecates at the same time Since the louse bite is irritating, the bitten person usually scratches and may thus rub the feces of the louse into the injured skin Probably this is the usual way in which typhus infection is passed from man to man It is also possible

on the sera of persons who have previously been vaccinated with killed rickettsial vaccines (Plotz and Wertman, 1945; Zarafonetis et al., 1946, Smadel, 1948). In recent years it has become increasingly difficult to obtain highly specific washed rickettsial suspensions for use in the complement-fixation test, since the commercial sources have discontinued the manufacture of the antigen

Antibodies which agglutinate suspensions of rickettsiae can be demonstrated in the sera of man and certain animals following infection with typhus The test requires more skill for its performance and is considerably more expensive than the complement-fixation test (cf Smadel, 1948).

Tests for the presence of opsonins, precipitins and neutralizing antibodies are valuable in the study of typhus, but are practicable only for laboratories in which extensive work with rickettsial diseases is in progress (1) precipitins, Lim and Kurotchkin (1929), Topping et al. (1945), (2) opsonins, Epstein (1922); Castaneda (1936a), (3) neutralization and protection tests, Gildemeister and Haagen (1940); Giroud (1938), Clavero and Perez Gallardo (1943a), Topping et al (1945); van den Ende et al. (1946)

A serologic test was developed by Chang in 1953 which is based on the agglutination of sheep or human group O erythrocytes after sensitization with a serologically active fraction derived by treatment of rickettsiae with ether, heat and alkali (Chang, 1953, Chang et al, 1953, 1954). The active material was designated the "erythrocyte sensitizing substance," or ESS. Erythrocytes exposed to the typhus ESS are specifically agglutinated by sera of patients as early as the beginning of the 2nd week of the illness The anti-ESS antibodies persist in the patients' sera for long periods after recovery from the disease. The typhus ESS is common to both epidemic and murine species and is distinct from the antigens involved in complement-fixation, rickettsial agglutination, or the Weil-Felix reaction. The typhus ESS may have a slight antigenic overlap with the ESS derived from members of the Rocky Mountain spotted fever group. The ESS test is simpler to perform and less expensive than the complement-fixation test.

The fluorescent antibody technic as modified by Goldwasser and Shepard (1958) may provide a useful procedure for the laboratory diagnosis of typhus

Serologic technics are the methods of choice in the diagnosis of louse-borne typhus, since attempts to recover *R. prowazeki* by inoculation of animals, eggs or insects involve procedures which require special laboratory facilities as well as skill in interpretation of results. Thus, in order to establish definitely that *R prowazeki* has been isolated from a patient, it is necessary to demonstrate the development of specific antibodies, the absence of cultivable bacteria, the existence of reciprocal cross-immunity with known typhus strains, and the occurrence of specific pathologic lesions with typical intracellular rickettsiae

Treatment

All persons who handle typhus cases should be immunized To prevent lice from gaining access to their clothing, they should protect themselves carefully during the delousing of patients by the use of rubber gloves and surgical gowns. On admission to a hospital, a patient should be bathed with soap and water or 1 per cent solution of Lysol, and his clothes should be promptly disinfected, preferably by heat. A patient and his hospital garments should be carefully dusted with 10 per cent DDT delousing powder on admission to the wards and once a week thereafter until discharge. In order to disinfect and delouse a patient, formerly it was required that his head and axillary and pubic regions be shaved, this is no longer necessary. After the disinfestation procedures are completed, ordinary precautions are adequate.

Skillful and diligent nursing is of great importance. Constant supervision is necessary to prevent a delirious patient from doing himself bodily harm Stuporous patients or those in coma should be moved frequently from side to side to prevent bed sores. A rise of body temperature to 40 5° C or above should be treated promptly by cold sponges Oral hygiene is very important in the prevention of parotitis. Fluids should be administered at frequent intervals in adequate quantity to produce at least 1,500 ml. of urine daily A liquid or semisolid diet high in caloric content and in vitamins is desirable. Paraldehyde and chloral hydrate are valuable in the con-

tients and their clothes is described above under Treatment.

These recommendations may be amplified in regard to immunization and louse control. Both of these procedures are applicable to an individual as well as to a community. Vaccines for protection against epidemic typhus are of 3 general sorts.

1. The preferred type of antityphus vaccine contains killed *R. prowazeki* which cannot cause typhus. Adequate quantities of rickettsiae for such vaccines may be obtained from the intestines of human lice (da Rocha-Lima, 1918, Weigl, 1930), from rodent lungs (Castaneda, 1939, Durand and Giroud, 1940), from tissue cultures (Zinsser et al, 1938), and from the yolk-sac membrane of developing chick embryos (Cox, 1938, 1941, 1948; Craigie, 1945, Topping et al, 1945). The last, known as the Cox-type vaccine, was produced on a tremendous scale during World War II and was administered to several million troops who were sent to areas where they might be exposed to epidemic typhus. Civilian populations in certain of the danger zones were also given the vaccine (Table 34). Cox-type vaccine probably reduces the incidence of the disease in a group of exposed persons, but there are no data with satisfactory controls on this point. However, it has been definitely established that it reduces the mortality from typhus to practically zero. The course of the disease in persons immunized with this vaccine is milder and shorter than is that in unvaccinated persons, furthermore, the incidence of serious complications is sharply reduced (Ding, 1943, Ecke et al, 1945, Gilham, 1946).

In immunized American troops in World War II there were only 64 patients with mild epidemic typhus, all of whom recovered (Sadusk, 1947). The official recommendations for the Cox-type vaccine are 2 subcutaneous doses of 1 ml. each, separated by an interval of from 10 to 14 days, followed by a booster dose of 1 ml at the beginning and in the middle of the typhus season (Sadusk, 1947). Before receiving yolk-sac vaccine, persons should be asked if they are sensitive to egg proteins. If so, the use of the vaccine should be undertaken only with caution, since severe

TABLE 34 EFFECTS OF COX-TYPE VACCINE ON EPIDEMIC TYPHUS, CAIRO, EGYPT, 1943 AND 1944

	NUMBER OF PATIENTS *	AVERAGE AGE, YEARS	AVERAGE DURATION OF FEVER, DAYS,	NUMBER OF DEATHS
No vaccine	47	26	18	9
Vaccinated †	20	32	11 6	0

* Figures refer to male Egyptian patients, ages 18-48, studied in U S A Typhus Commission ward in the Cairo Fever Hospital

† Vaccinated One or more doses of Cox vaccine, 1 cc each, more than 20 days before onset of illness Average amount of vaccine this group, 2 5 cc Average interval between last dose and onset, 2½ months

reactions may occur. Complement-fixing antibodies may persist for several years after a course of Cox-type typhus vaccine, furthermore, a booster dose of 1 ml induces a rapid serologic response in persons immunized several years before, even though they may not have had demonstrable antibodies at the time the booster dose was given (Murray, Ofstrock and Snyder, 1952).

2. The second type is a vaccine prepared from "Strain E," a variant of epidemic typhus. This was discovered during World War II (Clavero and Gallardo, 1943b), and has been studied extensively in the past decade (Fox et al, 1957). Several thousand people have been inoculated with Strain E. Trials on small groups have shown that Strain E can induce immunity in man to direct challenge with a virulent epidemic strain. The rickettsiae in the vaccine are living, but they have lost the property of causing serious illness in man. Reactions have occurred after inoculation of the minimum immunizing dose of Strain E vaccine, but the long duration (at least 5 years) of the immunity from a single dose is a feature which may be of overriding importance under certain circumstances. Studies are in progress to determine the efficacy of Strain E vaccine in protection against naturally occurring epidemic typhus in Peru (Fox et al, 1955).

3. The third type of vaccine is composed of living murine rickettsiae treated with cer-

TABLE 32 TYPHUS FEVER IN NAPLES, DECEMBER 1943 TO FEBRUARY 1944
(Van den Ende et al., 1946, p 35)

Years	Males		Females		Total		Mortality Percentage
	Cases	Deaths	Cases	Deaths	Cases	Deaths	
Under 3	20	1	18	0	38	1	2 6
3-11	133	2	91	1	224	3	1 3
12 20	221	9	166	10	387	19	4.9
21-29	105	11	108	10	213	21	9.8
30-38	111	14	127	18	238	32	13 4
39-47	59	29	116	28	175	57	32 5
48-56	43	18	56	19	99	37	36 3
57 65	10	7	27	15	37	22	59 4
66-74	2	2	8	3	10	5	50 0
75 and over	1	1	1	1	2	2	100 0
Gross	705	94	718	105	1423	199	13 9

TABLE 33. TYPHUS FEVER, CAIRO, EGYPT, JANUARY 1943 TO AUGUST 1944
(Ecke et al , 1945, p 451)

Ages	Males			Females		
	Cases	Deaths	Mortality Percentage	Cases	Deaths	Mortality Percentage
16-20	1247	120	9 6	689	60	8 7
21-25	1363	208	15 2	586	61	10 4
26-30	988	252	25 5	540	74	13 7
31-35	598	184	30 8	277	71	18 8
36-40	422	142	33.6	332	59	25 4
41-48	264	124	47 0	135	44	32.6

to become infected by crushing an infected louse on the skin, by rubbing infected louse feces into the eyes or by having dried infected louse feces gain access to the conjunctivae or to the mucous membranes of the respiratory tract. Once deloused and bathed, typhus patients are not capable of transmitting the infection to other persons by contact; *R prowazeki* does not occur in saliva, sputum, urine or feces of patients unless blood is also present With the exception of man, no other reservoirs of epidemic typhus are known

CONTROL MEASURES

The official recommendations of the American Public Health Association (1955, p. 205) should be followed by physicians who are faced with outbreaks of epidemic typhus "The most important measure for the rapid control of typhus, where the reporting has been good and the number of cases small, is the application of insecticides with residual effect to all contacts. Where the infection is known to be widespread, the systematic application of residual insecticide to all persons in the community is indicated." Immunization of persons in contact with cases is recommended; exposed susceptible persons, if louse infested, should be dusted with residual insecticide, quarantine is then unnecessary. The nearest World Health Organization Regional Epidemiological Information Station should be notified at once of the existence of a typhus epidemic. The disinfection of pa-

currence of wide swings, (3) the rash may be absent in many cases, (4) complications are much less frequent, except in persons of advanced age, and (5) the age-specific case-fatality rate is lower.

Pathologic Picture

Although the histopathology of Brill-Zinsser disease has not been studied extensively, the lesions which have been observed do not differ in any important respect from those found in the primary epidemic form of typhus fever

Experimental Infection, Host Range

The rickettsiae isolated from patients with Brill-Zinsser disease are indistinguishable from epidemic strains as regards experimental infection and host range (Murray and Snyder, 1951; Price et al, 1958)

Etiology

Extensive comparisons of 7 strains of Brill-Zinsser disease rickettsiae with standard epidemic and murine strains have established that this disease is caused by *R prowazeki*, not *R. mooseri* (cf Murray and Snyder, 1951, Price et al, 1958) It is erroneous to refer to "Brill's disease" as endemic or murine typhus, or to use the term "Brill's disease" to describe an illness transmitted to man by rat fleas (cf section on Murine Typhus)

Diagnosis

A diagnosis of Brill-Zinsser disease should be considered when a patient who has lived in a typhus zone develops a fever, a severe *intractable headache* and possibly a *macular* or maculopapular rash The diagnosis can be confirmed by the demonstration in serial serum specimens of a rising titer of antibodies which fix complement in the presence of epidemic typhus antigen or agglutinate suspensions of washed epidemic typhus rickettsiae There are two important differences between the serologic features of Brill-Zinsser disease and primary epidemic louse-borne typhus *The first difference is that in Brill-*Zinsser disease the rise in specific antibodies begins early, on the 4th to the 6th day after the onset of illness, the peak response is usually attained between the 8th and the 10th days (Murray et al, 1951) In the primary attack of epidemic typhus, on the other hand, the specific antibody rise begins later, about the 8th to the 12th day with maximum titers being reached from the 12th to the 16th day after onset (Plotz et al, 1948) *The second* important serologic difference is that the Weil-

Table 35 Differences Between Primary Attacks and Recrudescences of Epidemic Typhus

Primary Epidemic Louse-Borne Typhus		Brill-Zinsser Disease
No	History of Previous Attack of Typhus	Yes
Epidemic	Occurrence	Sporadic
Transmitted by human body lice	Relation to Lice	Can occur in absence of lice
12 to 18 days	Usual Duration of Fever	7 to 11 days
	Complement-Fixation Test	
Less than 1/100	(a) Titer on 8th day of illness	More than 1/1,000
Later than 12th day	(b) Maximum titer occurs	*Between 8th and 10th day*
	Weil-Felix Test	
Usually from 1/320 to 1/5,000	Maximum titers	Usually less than 1/160

tain agents in an effort to attenuate the organisms (Laigret and Durand, 1939; Blanc and Baltazard, 1941). These living vaccines have been used on a very large scale for immunization of natives in French North Africa (cf. Biraud, 1943), but their use is not without danger, since they cause attacks of murine typhus. deaths have been recorded (Palacios et al , 1935, Sadusk and Kuhlenbeck, 1946)

The efficacy of delousing in controlling epidemics was shown in North Africa in 1912 (Otto and Munter, 1930) and in Serbia in 1915 (Strong et al , 1920) At that time and until very recently, in order to be deloused it was necessary for infested persons to remove all clothes, which were then subjected to heat while they bathed When large groups were involved, this process was very cumbersome, expensive and time-consuming

The disadvantages of delousing by heat were eliminated in 1943 when various antilouse powders were developed and methods devised (Wheeler, 1943, Soper et al , 1945) whereby large numbers of people could be treated without removing their garments The powders were shown to be effective when blown into the hair, up the sleeves, down the neck and around the waist into trousers The now famous insecticide, DDT, dichloro-diphenyl-trichloro-ethane (Mooser, 1942; Bishopp, 1945, Knipling, 1948), proved to be nearly ideal

The most satisfactory property of DDT is the persistence of its lethal effect on lice for more than 2 weeks after being dusted into the garments, or for more than 4 weeks after impregnation from an emulsion Reinfestation of dusted persons was reduced to a negligible degree by this persisting effect of DDT. The final improvement in delousing technic was the development of a power duster, a device consisting of an air compressor which operates 10 dust guns simultaneously, the technic has been described by Greeley (1948)

Recent reports indicate that human body lice in several areas have become partially or highly resistant to DDT (Hurlbut et al., 1954, Eddy et al , 1955, and Wright and Brown, 1957). In such circumstances insecticides, preferably with residual effect, must be used for effective louse control (Cole and Burden, 1956).

BRILL-ZINSSER DISEASE (RECRUDESCENT TYPHUS)

(Synonym: Sporadic typhus)

Introduction

Brill-Zinsser disease is a recrudescence of epidemic louse-borne typhus fever in relatively mild form years after the primary attack

History

In 1898, Brill noted the occurrence in New York City of a disease resembling typhus fever Cases continued to appear sporadically in that city and elsewhere No association with human body lice could be established Anderson and Goldberger (1912) showed that the disease was a form of typhus by transmitting the infection to monkeys and by

parts of eastern Europe. However, in the period preceding the recognition of murine flea-borne typhus, all sporadic cases of typhus in the United States were customarily referred to as Brill's disease; this fact resulted in much confusion (cf section on Murine Flea-borne Typhus).

Zinsser (1934), after scrutinizing the records of 538 cases of "Brill's disease," advanced his hypothesis that the disease is a recrudescence of epidemic typhus in persons who suffered an attack of the classic disease many years previously while residing in areas where epidemics of typhus were occurring Zinsser postulated the persistence of the typhus rickettsiae during the latent interval somewhere in the tissues of the human subject Zinsser's hypothesis has been verified in large part by recent studies The term Brill-Zinsser disease was proposed by Loeffler and Mooser in 1952 in recognition of Zinsser's appreciation of the epidemiologic significance of the disease.

Clinical Picture

The factors which precipitate Brill-Zinsser disease are not known, consequently, there are no data on the incubation period. Clinical features are those of epidemic louse-borne typhus with the following exceptions: (1) Brill-Zinsser disease is shorter in duration (7 to 11 days); (2) the temperature curve is more irregular with a tendency for the oc-

currence of wide swings; (3) the rash may be absent in many cases; (4) complications are much less frequent, except in persons of advanced age; and (5) the age-specific case-fatality rate is lower

Pathologic Picture

Although the histopathology of Brill-Zinsser disease has not been studied extensively, the lesions which have been observed do not differ in any important respect from those found in the primary epidemic form of typhus fever

Experimental Infection, Host Range

The rickettsiae isolated from patients with Brill-Zinsser disease are indistinguishable from epidemic strains as regards experimental infection and host range (Murray and Snyder, 1951, Price et al, 1958)

Etiology

Extensive comparisons of 7 strains of Brill-Zinsser disease rickettsiae with standard epidemic and murine strains have established that this disease is caused by *R prowazeki*, not *R mooseri* (cf Murray and Snyder, 1951, Price et al, 1958) It is erroneous to refer to "Brill's disease" as endemic or murine typhus, or to use the term "Brill's disease" to describe an illness transmitted to man by rat fleas (cf section on Murine Typhus)

Diagnosis

A diagnosis of Brill-Zinsser disease should be considered when a patient who has lived in a typhus zone develops a fever, a severe intractable headache and possibly a macular or maculopapular rash The diagnosis can be confirmed by the demonstration in serial serum specimens of a rising titer of antibodies which fix complement in the presence of epidemic typhus antigen or agglutinate suspensions of washed epidemic typhus rickettsiae There are two important differences between the serologic features of Brill-Zinsser disease and primary epidemic louse-borne typhus The first difference is that in Brill-Zinsser disease the rise in specific antibodies begins early, on the 4th to the 6th day after the onset of illness, the peak response is usually attained between the 8th and the 10th days (Murray et al, 1951) In the primary attack of epidemic typhus, on the other hand, the specific antibody rise begins later, about the 8th to the 12th day with maximum titers being reached from the 12th to the 16th day after onset (Plotz et al, 1948) The second important serologic difference is that the Weil-

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	Weil-Felix Test	
Usually from 1/320 to 1 5,000	Maximum titers	Usually less than 1/160

Felix reaction (agglutination of Proteus OX 9) usually is negative in Brill-Zinsser disease (Murray et al, 1951).

The differences between primary epidemic typhus and Brill-Zinsser disease are summarized in Table 35 (modified from Murray and Snyder, 1953).

At least 30 per cent of persons who formerly lived in an epidemic typhus area can be expected to have residual antityphus complement-fixing and neutralizing antibodies, even though 40 to 50 years may have elapsed since their last possible exposure to typhus (Murray et al, 1952; Price et al, 1958). Thus, a definite rise in titer of more than 4-fold in the complement-fixation test with epidemic typhus antigen must be demonstrated in association with a particular illness in such persons in order to establish a diagnosis of Brill-Zinsser disease by serologic procedures.

Antibodies which fix complement in the presence of murine typhus antigen may also develop during the disease However, the maximum titers are regularly lower than those with epidemic antigen when the highly specific washed rickettsial suspensions are used as test antigens (Murray et al, 1950).

Treatment

The tetracyclines or chloramphenicol can be expected to exert a beneficial effect on Brill-Zinsser disease, although the evaluation of such therapy is more difficult because of the relatively short and mild course of the untreated disease

Epidemiology

Brill-Zinsser disease has been recognized in persons who migrated to several countries in which epidemic louse-borne typhus is not present, namely, the United States (Brill, 1910; Zinsser, 1934; Morgan et al., 1948; and Murray et al., 1950), Switzerland (Mooser and Loeffler, 1946), England (Hawksley and Stokes, 1950), France (Giroud et al., 1950, Worms, 1950) Portugal (Soares, 1950), and Poland (Kostrzewski et al., 1957). The illness is by no means restricted to that portion of a population which has migrated away from a typhus zone In a very brief interval in the summer of 1950, Murray and his associates identified 26 cases in a relatively small part of Yugoslavia, an area which had been swept over by louse-borne typhus in 1944-45 (Murray et al, 1951). These cases were about equally distributed between males and females; the age range was 9 to 56 years; there were no secondary cases; there was no association with human body lice, nearly all of the patients gave a clear history of a previous attack of typhus which occurred during the louse-borne epidemics of 1944-45. Only one of the cases was sufficiently like epidemic typhus to appear in the official case reports These observations indicate several features of importance epidemiologically:

(1) Brill-Zinsser disease can be expected to occur in the native population of typhus zones as well as in persons who migrate to nontyphus zones; (2) the disease definitely is not restricted to any one race, sex or age group; (3) the incidence of Brill-Zinsser disease may be considerably greater than formerly suspected; (4) many of the hundreds of thousands of displaced persons who recently have settled in nontyphus zones are notorious concentration camps (Snyder, 1947); (5) Brill-Zinsser disease probably will not be recognized unless physicians are alert to the mild course, the frequent absence of a rash, and the negative Weil-Felix reaction

No clues have been obtained as to factors which precipitate the recrudescence of typhus It is believed that after a person recovers from primary louse-borne typhus the rickettsiae remain viable somewhere in his tissues. The balance between the immune mechanism and the parasite is disturbed somehow, and a recrudescence occurs The early serologic response in Brill-Zinsser disease and its milder course strongly suggest that partial immunity is still present. Price (1955) isolated virulent epidemic typhus rickettsiae from the lymph nodes of two "healthy" subjects who had resided in a typhus zone early in their lives but who had been continuously in an environment believed to be completely free of epidemic typhus for more than a decade prior to the recovery of the organisms from their lymph nodes Both of these subjects had low-titer antibodies in their sera, indicating previous typhus infection. Neither subject had clinical signs or symptoms suggestive of active infection

fered this suggestion, predicting that human body lice might become infected if they fed upon patients with Brill-Zinsser disease. This has now been conclusively demonstrated, 7 patients studied in the United States were infectious for lice which were allowed to feed on them at some time before the 8th day of the illness (Murray et al, 1950) The typhus rickettsiae in these lice were fully virulent, indistinguishable from the classic strains (Murray and Snyder, 1951) Thus, under disturbed conditions in a community which permit the development of louse infestation, patients with Brill-Zinsser disease may be the foci from which an epidemic of louse-borne typhus fever may spread to susceptible persons in the population Murray and his associates in Yugoslavia (1958) have identified several small outbreaks of louse-borne typhus which clearly originated in a case of Brill-Zinsser disease The differentiation was accomplished on the basis of the distinctive serologic patterns the very rapid rise in complement-fixing antibodies early in Brill-Zinsser disease permits identification of the recrudescent cases from the primary louse-borne cases The fact that the outbreaks noted by Murray remained small and sharply localized is compatible with absence of overcrowding and with relatively low rates of louse infestation in the areas at the time of the studies

Control Measures

There are no control measures which are known to be effective in reducing the incidence of Brill-Zinsser disease If such cases occur in the presence of louse infestation, the situation should be regarded potentially as the beginning of an epidemic, and those measures described for the control of epidemic louse-borne typhus should be applied

MURINE TYPHUS (FLEA-BORNE)

(Synonyms Endemic typhus, urban or shop typhus of Malaya, flea typhus, rat typhus)

Introduction

Murine typhus is a relatively mild, acute febrile illness of 9 to 15 days' duration, characterized by headache and macular rash It is a natural infection of rats and mice transmitted sporadically to man by the rat flea, *Xenopsylla cheopis* The etiologic agent is *Rickettsia mooseri* (Monteiro, 1931) The case-fatality rate for all ages is approximately 2 per cent.

History

Murine typhus probably has occurred since ancient times, but only in recent years has it been differentiated from epidemic louse-borne typhus Although sporadic cases of typhus in Europe had been referred to occasionally (McCrae, 1907), such reports received little notice In the United States, sporadic cases of typhus were noted by Brill (1910), Lee (1912) and Paullin (1913) Hone (1922) described several isolated cases in Australia, and Wheatland (1926) called attention to the occurrence of a noncontagious typhuslike fever among the farm population in Queensland, at a time when a plague of mice afflicted the region Sinclair and Maxcy (1925) and later Maxcy (1926, 1929) investigated cases of "endemic" typhus in southeastern United States On epidemiologic grounds, Maxcy (1929) postulated that a reservoir of the disease other than man exists and mentioned that rats and mice might serve as such a reservoir He also mentioned that fleas, mites or ticks might be the vectors The epidemiologic evidence for postulating a separate variety of typhus was strengthened by observations of differences in the pathologic features (Wolbach and Todd, 1920) and in the characteristics of strains isolated from the sporadic cases of typhus in Mexico Neill (1917) observed that male guinea pigs inoculated with typhus strains obtained from Mexico exhibited enlargement of the scrotal sac and adhesions of the testes Mooser (1928) reported that certain strains of typhus rickettsiae isolated from patients in Mexico not only caused the tunica reaction in male guinea pigs but also multiplied profusely in the serosal cells over the testes The tunica reaction of guinea pigs has been referred to as the "Neill-Mooser reaction" and the cells packed with rickettsiae as "Mooser cells" The final steps proving the existence of a second variety of typhus were made by Dyer, Rumreich and Badger (1931) who isolated rickettsiae from rat fleas in Baltimore, and by Mooser, Castaneda and Zinsser (1931a) who found the typhus agent in brains of rats trapped in Mexico City. The disease was then named murine typhus to indicate its presence as a natural infection of rats (Mooser, 1932) It was promptly sought for and found in most parts of the world (Biraud and Deutschman, 1936) Biologic differences between typhus strains isolated from rats or

fleas and those isolated from patients suffering from classic louse-borne epidemic typhus were demonstrated by Zinsser and his colleagues (Zinsser, 1940, Mooser, 1945). The two varieties of typhus were further characterized by application of serologic technics (Smadel, 1948) and by cross-vaccination experiments (Topping et al, 1945; Craigie et al, 1946, Murray and Snyder, 1951).

Clinical Picture

Murine typhus in man is similar to epidemic louse-borne typhus In the absence of epidemiologic and laboratory data, it is impossible on the basis of clinical findings alone to determine whether a patient is suffering from murine typhus instead of epidemic typhus However, murine typhus is relatively mild with a negligible mortality except in persons more than 50 years old The onset is likely to be more gradual than that of epidemic typhus, the symptoms less severe, the rash shorter in duration, and the skin lesions less numerous The central nervous system, the myocardium and the kidneys are involved less severely. Complications, e g, parotitis, necrosis of the skin, gangrene of the extremities, otitis media, furunculosis, azotemia and bronchopneumonia, occur infrequently The temperature curve usually shows wider fluctuations than that of epidemic typhus The febrile period is terminated by rapid lysis after 9 to 14 days Recovery is prompt without sequelae Laboratory data are similar to those described for epidemic typhus, except that abnormal findings tend to be slight or absent in murine typhus Clinical features are described in detail by Maxcy (1926), Miller and Beeson (1946), and Woodward (1948)

Pathologic Picture

The pathology of murine typhus in man probably is similar to that of epidemic typhus However, there is a paucity of data on this point, since very few postmortems have been reported in cases of proved murine typhus.

Experimental Infection; Host Range

Murine typhus occurs as a natural infection of rats and mice. It is transmitted from rat to rat either by the rat louse, ***Polyplax spinulosus*** (Mooser, Castaneda and Zinsser, 1931b), or by the rat flea, ***Xenopsylla cheopis*** (Dyer, Rumreich and Badger, 1931). Transmission from rat to man is attributed to *X* ***cheopis*** The infection in man is an accidental occurrence, unconcerned in the maintenance of the infection in nature Some observers (Zinsser, 1937) regard the present evidence sufficient to establish the fact that murine typhus may be spread from man to man in epidemic form by the human body louse (Mooser, 1945). Many species in addition to those mentioned are susceptible to murine typhus: monkeys, donkeys, cats, squirrels, deer mice, voles, gerbilles, cotton rats, guinea pigs, developing chick embryos, cat fleas, tropical rat mites, etc ; see articles by Wolbach (1940) and Philip (1948)

The rat flea, *X.* ***cheopis,*** becomes infected by feeding on a rat which is in the acute phase of an infection The rickettsiae multiply in the cells of the flea without causing damage to the host. Once infected, the flea continues to discharge rickettsiae in its feces for the duration of its life. Other species of fleas, notably ***Pulex irritans,*** are susceptible, but their reaction to typhus infection has not been thoroughly studied When human body lice are experimentally infected with murine typhus, the resulting disease is similar in all respects to the infections caused by epidemic strains (Mooser, 1945) Whether or not lice acquire murine typhus under natural conditions is open to question, experimental passage of 2 strains of murine typhus serially in human body lice for several transfers was not accompanied by any change in characteristics toward epidemic strains (Murray and Snyder, 1951)

Murine typhus can be established in guinea pigs by intra-abdominal inoculation of the blood of a patient by the method described for epidemic typhus The infection, once established in serial passage in these animals, has an incubation period of from 3 to 7 days The temperature curve often exhibits fluctuations but tends to stay at or above 40° C. for several days. The fever is usually accompanied by enlargement of the scrotal sac of male guinea pigs, reddening of the skin of the scrotum, and adhesions between testes and tunica vaginalis This "tunica reaction" is quite distinct from the more severe "scrotal reaction" caused by spotted fever. In the latter, necrosis of the scrotal skin often occurs. When an animal is sacrificed on the 1st or

2nd day of the tunica reaction, two phenomena are observed at necropsy the spleen is covered by a thin coat of fibrin which may cause the spleen to adhere to the abdominal wall, and the testes may show adhesions and a few small subserosal hemorrhages Smear preparations from the surface of the testes, the peritoneum, or the spleen contain numerous rickettsiae, intracellular and extracellular in location Transfer to other guinea pigs is accomplished by removing the spleen, grinding it in a mortar with sterile sand or alundum to a 10 per cent suspension in a suitable diluent, after gross particles are removed by light centrifugation, the supernatant suspension in 1 or 2 ml. amounts is inoculated intra-abdominally into fresh normal male guinea pigs An alternate method of transfer is the use of a suspension obtained by shaking the testes in a flask with glass beads and 20 to 30 ml of a suitable diluent The materials prepared in this manner on the 1st or 2nd day of the tunica reaction contain at least 10^6 viable rickettsiae per ml It is a common observation that during the hot summer months, murine typhus tends to lose its distinguishing features in guinea pigs, but in the cold months the fever and tunica reaction reappear Great reliance was formerly placed on the tunica reaction of guinea pigs as a means of differentiating between the epidemic and the murine varieties More extensive experience has shown that occasionally epidemic strains may produce the tunica reaction, and conversely, that a murine strain may fail to cause it Specific serologic tests and cross-vaccination experiments now provide such reliable and rapid differentiation between epidemic and murine strains that the tunica reaction of guinea pigs is no longer relied upon to distinguish one variety from the other

Murine typhus may be maintained indefinitely by passage in white rats, whereas epidemic strains die out after a few passages Murine rickettsiae usually multiply profusely in the cells of the peritoneum and may cause fever and scrotal swelling, but the animals recover as a rule An important feature of the murine infection in rats is the persistence of viable rickettsiae in the brain for several months (Philip, 1948) Large doses of *R mooseri* may produce death of white mice within a few hours as a consequence of their toxic properties (Gildemeister and Haagen, 1940) Slightly smaller doses cause extensive peritonitis with death of the animals in 3 to 8 days Enormous numbers of rickettsiae are found in the peritoneal exudate of moribund mice. Inocula containing relatively few *R mooseri* render mice immune with 3 weeks to toxic and infective doses of the organism Rats and mice, as well as sheep, dogs and rabbits, may be infected by the intranasal route and succumb in a few days with extensive pneumonitis This procedure has been utilized for vaccine production Irradiation of white rats and cotton rats with deep x-rays greatly increases the yield of murine typhus rickettsiae in these animals

Etiology

The causative agent of murine typhus has been named *Rickettsia mooseri* (Monteiro 1931) in honor of Mooser and is similar to *R prowazeki* as regards size shape, staining properties, and resistance to chemical and physical agents but exhibits less pleomorphism Antigenic composition has been described in the section on etiology under epidemic typhus *R. mooseri* has been cultivated in the yolk-sac membrane of developing chick embryos with considerable success (Cox, 1938)

Diagnosis

Epidemic typhus and murine typhus cannot be distinguished solely on clinical grounds in individual cases Since murine typhus may exist in the same regions with Rocky Mountain spotted fever (United States, Mexico, South America, probably India), it may be emphasized that the rash of typhus appears on the body first, then spreads to the extremities, whereas in spotted fever the reverse is true The rash of the latter disease has a greater tendency to be papular and to become petechial or hemorrhagic Isolation of *R mooseri* can be accomplished by inoculation of white rats or guinea pigs with the blood of a patient Comments in the section on epidemic typhus in regard to use of ground clot apply equally well to murine typhus Direct isolation of murine typhus strains by inoculation of human blood into developing chick embryos, although entirely feasible, has not been reported The Weil-Felix reaction occurs in murine as well as in epidemic typhus.

TABLE 36 MURINE TYPHUS IN THE UNITED STATES

YEAR	CASES *	DEATHS †
1931	332	22
1936	1,732	112
1941	2,780	135
1946	3,365	128
1951	378	16
1956	98	Not Available

* Cases have been reported from more than three fourths of the states In the entire United States, from 1931 through 1946, approximately 42,000 cases were reported

† Figures taken from Supplements to Public Health Reports, U S Pub Health Service

The complement-fixation test, the agglutination of rickettsiae, and the neutralization of toxic properties have been discussed in the section on epidemic typhus. There are no important differences between the 2 types of typhus in regard to the time of appearance, peak of occurrence, or persistence of the various antibodies Sera of animals and man in convalescence from attacks caused by proved murine typhus strains usually have higher titers against homologous than against heterologous antigens Sera of patients who contract murine typhus after having received killed vaccine of the epidemic type may show no difference in titer when tested against the 2 antigens (Plotz and Wertman, 1945; Zarafonetis et al, 1946)

TREATMENT

Treatment of murine typhus differs in no important respect from that described for epidemic typhus The mildness of the disease and its slower onset tend to delay its clinical recognition until the optimum period for administration of antibiotics has passed

EPIDEMIOLOGY

Murine typhus is world-wide in distribution. In the United States, the reported cases from 1931 to 1956 (Table 36) indicate an increase in prevalence which seems to be of greater magnitude than can be accounted for on the basis of improved diagnosis In the years 1948 to 56, however, a sharp decline occurred in the reported cases of murine typhus in the United States Maximum incidence occurs in the summer and fall months

in part to difficulty in recognizing the rash in dark-skinned individuals Persons of both sexes are equally susceptible to the illness. The most important factor influencing the occurrence of murine typhus in human beings is residence or occupation in areas where rats abound. Although the disease often involves only one member of a household, it may appear in several occupants of a dwelling The usual manner in which the infection is transmitted to man is as follows: at the time an infected flea sucks blood, it deposits feces which may be scratched into the wound made by the bite. There are other possible modes of infection, for example, infected flea feces may gain access to the conjunctivae or mucous membranes of the respiratory tract, experiments with volunteers have shown that murine typhus can be contracted by the ingestion of viable *R. mooseri* (Pollard et al, 1946); it is possible that cases may be caused by eating food recently contaminated by the urine of infected rats. A patient suffering from murine typhus cannot transmit the infection to other persons by contact; *R. mooseri* does not occur in the sputum, the feces or the urine unless gross blood is also present. An attack of the disease results in immunity which persists for many years.

As knowledge of the differences between epidemic typhus and murine typhus has accumulated, it has become evident that both types are present in Mexico, and that epidemic strains are frequently isolated in that country from patients (Varela and Zozaya, 1945) Moreover, complement-fixation tests on sera of residents of Mexico indicate the preponderance of the classic infection in that region (Silva-Goytia, 1944) Therefore, the term "Mexican typhus" is ambiguous and should be avoided.

The relationship of murine to epidemic typhus has been the subject of much speculation One theory postulates that murine typhus is the more ancient disease as shown by the fact that the two principal hosts of *R. mooseri*, the rat and the rat flea, undergo no harmful effects from their intimate association with it. By contrast, *R. prowazeki* causes a serious illness in man and a fatal infection in the louse. These facts have been taken as evidence that, from the evolutionary viewpoint, the association of man and human lice with typhus rickettsiae is relatively recent.

Control Measures

The official recommendations of the American Public Health Association (1955, pp 206-7) are as follows:

A. Preventive measures

1. Application of insecticide powders with residual activity (10% DDT or other compounds) to rat runs, burrows, and harborages

2. Rodent control measures should be delayed until flea populations have been reduced by insecticides, to avoid temporary increase in cases

B Control of the infected individual, contacts and environment

1. Report to local health authority. Case report obligatory in most states and countries
2. Isolation. None
3 Concurrent disinfection None
4. Terminal disinfection None
5 Quarantine None

[illegible]

6 Specific treatment As for epidemic typhus

C Epidemic measures In endemic areas with numerous cases, widespread use of DDT has markedly reduced the flea index of rats, and incidence of infection in rats and man Inoculation with an inactivated *R mooseri* vaccine may be useful for limited groups in hazardous occupations but the efficiency of specific therapy eliminates the need for general protection of populations

D. International measures None

Measures for protection of individuals against murine typhus, such as the wearing of flea-proof garments, are impracticable Vaccines containing killed *R mooseri* afford protection in laboratory tests, but it is debatable whether vaccination of man is advisable *as a control measure, except for persons* who are frequently exposed to the infection More data are needed in regard to the efficacy of murine vaccine for human use It should be emphasized that, although an attack of epidemic typhus protects against murine typhus and vice versa, vaccines made from dead rickettsiae satisfactorily protect only against homologous strains To achieve protection against murine typhus, vaccines should be made with *R mooseri*, not *R prowazeki*. Considerable progress in the control of murine typhus on a community-wide basis has been made in three directions (1) ratproofing of buildings; (2) reducing flea populations through the use of DDT on rat runs; and (3) poisoning of rats with alpha naphthyl thiourea, warfarin (Hayes and Gaines, 1950) and other rodenticides The use of DDT should precede rat-poisoning campaign in order to kill potentially infected fleas before their hosts are poisoned Otherwise there may be a temporary increase in cases of murine typhus (Davis, 1947, Bradley and Wiley, 1948)

REFERENCES

(For complete references, see Chapter 35, 2nd edition)

American Public Health Association, 1955, The Control of Communicable Diseases, ed 8, New York, Am Pub Health Assn

Anderson, J F, and Goldberger, J, 1912, Collected Studies on Typhus Hygienic Lab Bull, No 86, Washington, D C

Biraud, Y., 1943, The present menace of typhus fever in Europe and the means of combating it, Bull Health Organ, League of Nations 10, 1-64

Biraud, Y, and Deutschman, S, 1936, Typhus and typhus-like rickettsia infect... [illegible]

[illegible]

...445-480

Bovarnick, M R, and Miller, J C., 1950, Oxidation and transamination of glutamate by typhus rickettsiae, J Biol Chem *184*, 661-676

Bovarnick, M R, Miller, J C, and Snyder, J C, 1950, The influence of certain salts, amino acids, sugars, and proteins on the stability of rickettsiae, J Bact *59*, 509-522

Bovarnick, M R, and Snyder, J C, 1949, Respiration of typhus rickettsiae, J Exper Med *89*, 561-565

Bradley, G., and Wiley, J S., 1948, The control of murine typhus in the United States Symposium on Rickettsial Diseases, Dec 1946, Boston, American Association for the Advancement of Science

Brill, N E., 1910, An acute infectious disease of unknown origin A clinical study based on 221 cases Am J M Sc *139*, 484-502

Castaneda, M R, 1934, The antigenic relationship between proteus X-19 and typhus rickettsia II A study of the common antigenic factor J Exper Med *60*, 119-125

———, 1936a, Studies on the mechanism of immunity in typhus fever III Demonstration of opsonins for *Rickettsia prowazeki* in typhus-immune serum J Immunol *31*, 227-236

TABLE 36 MURINE TYPHUS IN THE UNITED STATES

YEAR	CASES *	DEATHS †
1931	332	22
1936	1,732	112
1941	2,780	135
1946	3,365	128
1951	378	16
1956	98	Not Available

* Cases have been reported from more than three fourths of the states In the entire United States, from 1931 through 1946, approximately 42,000 cases were reported

† Figures taken from Supplements to Public Health Reports, U S Pub Health Service

The complement-fixation test, the agglutination of rickettsiae, and the neutralization of toxic properties have been discussed in the section on epidemic typhus. There are no important differences between the 2 types of typhus in regard to the time of appearance, peak of occurrence, or persistence of the various antibodies. Sera of animals and man in convalescence from attacks caused by proved murine typhus strains usually have higher titers against homologous than against heterologous antigens Sera of patients who contract murine typhus after having received killed vaccine of the epidemic type may show no difference in titer when tested against the 2 antigens (Plotz and Wertman, 1945; Zarafonetis et al., 1946).

TREATMENT

Treatment of murine typhus differs in no important respect from that described for epidemic typhus The mildness of the disease and its slower onset tend to delay its clinical recognition until the optimum period for administration of antibiotics has passed

EPIDEMIOLOGY

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Giroud, P., 1938, Essai de mise en évidence des anticorps du typhus exanthématique par un test cutané, Compt rend Soc biol *127*, 397-399

Giroud, P., Boyer, J., and Vargues, R., 1950, A propos de quelques cas de typhus exanthématique survenus chez des sujets non parasites, Presse med *58*, 334-336

Goldberger, J., and Anderson, J F., 1912, The transmission of typhus fever, with especial reference to transmission by the head louse (*Pediculus capitis*), Pub Health Rep *27*, 297-307

Goldwasser, R A., and Shepard, C C., 1958, Staining of complement and modifications of fluorescent antibody procedures, J Immunol *80*, 122-31

Greeley, D., 1948, Methods of application of DDT Symposium on Rickettsial Diseases, Dec 1946, Boston, American Association for the Advancement of Science

Haddon, W., Jr., 1956a, An artificial membrane and apparatus for the feeding of the human body louse *Pediculus humanus corporis*, Am J Trop Med *5*, 315-25

———, 1956b, The maintenance of the human body louse Pediculus humanus corporis through complete cycles of growth by serial feeding through artificial membranes, Am J Trop Med *5*, 326-30

Hamilton, H L., 1945, Specificity of the toxic factors associated with the epidemic and the murine strains of typhus rickettsiae, Am J Trop Med *25*, 391-395

Hawksley, J C., and Stokes, E J., 1950 A case of Brill's disease in London, Lancet *2*, 97-99

Hayes, W J., and Gaines, T B 1950, Control of Norway rats with residual rodenticide warfarin Pub Health Rep *65*, 1537-1555

Hone, F S., 1922, A series of cases closely resembling typhus fever, M J Australia *1*, 1-13

Hopps, H E., Hahn, F E., Wisseman, C L Jr., Jackson, E B., and Smadel, J E., 1956 Metabolic studies of rickettsiae III Studies of transamination, oxidative phosphorylation and glutamate-2-C[14] incorporation by purified suspensions of *Rickettsia mooseri* J Bact *71*, 708-16

Hurlbut, H S., Peffly, R L., and Salah, A A., 1954, DDT resistance in Egyptian body lice, Am J Trop Med *3*, 922-9

Kostrzewski, J., Gruzewski, A., and Milewska, L., 1937, Further remarks on the possible anticipation of recrudescences of typhus, Przeglad Epidem *11*, 21-26 From T D Bulletin 1957, *54*, 801

Laigret, J., and Durand, P., 1939, La vaccination contre le typhus *exanthématique Nouvelle technique* de preparation du vaccin emploi des cerveaux de souris Bull Acad med *122*, 84-89

Lee, R I., 1913, Typhus fever (Brill's disease) at the Massachusetts General Hospital in ten years (Oct 1, 1902, to Oct 1, 1912) Boston Med & Surg J *168*, 122-127

Ley, H R., and Smadel, J E., 1954, Antibiotic therapy of rickettsial diseases Antibiotics & Chemother *4*, 792-802

Lim, C E., and Kurotchkin, T J., 1929, A precipitin test for typhus fever with specific substance of *Bacillus proteus X 19*, Nat Med J China *15*, 6-10

Loeffler, W., and Mooser, H., 1952, Ein weiterer Fall von Brill-Zinsserscher Krankheit in Zurich; Spater Ruckfall bei klassischem Fleckfieber, Schweiz med. Wchnschr *82*, 493

MacArthur, W P., 1954, The athenian plague a medical note, Classical Quarterly *4* (n.s.), Nos 3-4, 171-174

Macchiavello, A 1937, Estudios sobre tifus exantematico III Un nuevo metodo para tenir Rickettsia Rev chilena hig *1*, 101-106

McCrae, T., 1907 Typhus fever in Osler, Modern Medicine vol *2*, p *232*, Philadelphia Lea & Febiger

Maxcy K F., 1926, Clinical observations on endemic typhus (*Brill's disease*) in the United States, Pub Health Rep *41*, 1213-1220.

———, 1929, Typhus fever in the United States, Pub Health Rep *44*, 1735-1742

Miller, E S., and Beeson, P B., 1946, Murine typhus fever Medicine *25*, 1-15,

Monteiro J L., 1931, Estudos sobre o typho exantematico de São Paulo, Mem Inst Butantan *6*, 1-135

Mooser H., 1928, Experiments relating to the pathology and the etiology of Mexican typhus (tabardillo) J Infect Dis *43*, 241-272

——— 1932 Essai sur l'histoire naturelle du typhus exanthematique Arch Inst Pasteur de Tunis *21*, 1-19

———, 1942, Le typhus exanthematique Cours de med et chir de guerre Genève, sept, Q G du I er corps d'armée [Quoted from Mooser, 1946]

———, 1945, Die Beziehungen des murinen Fleckfiebers zum klassischen Fleckfieber, Acta Tropica (Suppl) 4 Basel Reinhardt.

——— 1946, Twenty years of research in typhus fever Schweiz med Wchnschr *76*, 877-882

Mooser H., Castaneda M R., and Zinsser, H., 1931a Rats as carriers of Mexican typhus fever, J.A.M.A *97* 231-232

——— 1931b, The transmission of Mexican typhus from rat to rat by *Polyplax spinulosus*, J Exper Med *54*, 567-575

Mooser, H., and Loeffler, W., 1946, Ein Fall Sogenannter Brillscher Krankheit in Zurich, Schweiz med. Wchnschr *76*, 150-153

Morgan H R., Neva, F A., Fahey, R J., and Finland, M 1948, Report of two serologically proved cases of typhus fever in Irish-born residents of Boston, New England J Med *238*, 871-873

Murchison, C., 1884, A Treatise on the Continued Fevers of Great Britain, ed *3*, London, Longmans, Green

Murray, E S., Baehr, G., Shwartzman, G., Mandelbaum, R A Rosenthal, N., Doane, J C., Weiss, L B., Cohen S., and Snyder, J C., 1950, Brill's disease I Clinical and laboratory diagnosis J.A.M.A *142*, 1059-1066

Murray, E S., Cohen, S., Jampol, J., Ofstrock, A., and Snyder, J C., 1952, Epidemic typhus antibodies in human subjects in Boston, Mass., New England J Med *246*, 355-359

Castaneda, M R, 1936b, Studies on the mechanism of immunity in typhus fever Complement-fixation in typhus fever J Immunol *31*, 285-91

———, 1939, Experimental pneumonia produced by typhus rickettsiae, Am J Path *15*, 467-475

Chang, [redacted] M, Murray, E S, and Snyder, J C, 1954, Erythrocyte sensitizing substances from rickettsiae of the Rocky Mountain spotted fever group, J Immunol *73*, 8-15

Chang, S M, 1953, A serologically active erythrocyte-sensitizing substance from typhus rickettsiae. I Isolation and titration J Immunol *70*, 212-14

Chang, [redacted] M, Snyder, J C, and Murray, E S, 1953, A serologically active erythrocyte-sensitizing substance from typhus rickettsiae II Serological properties J Immunol *70*, 215-21

Clarke, D H, and Fox, J P, 1948, The phenomenon of *in vitro* hemolysis produced by the rickettsiae of typhus fever, with a note on the mechanism of rickettsial toxicity in mice, J Exper Med *88*, 25-41

Clavero, G, and Perez Gallardo, F, 1943, Técnicas de laboratorio en el tifus exantemático, Madrid, Prensa española

———, 1943b, Estudio experimental de una cepa apatogena e immunizante de *Rickettsia prowazeki* cepa E, Rev san e hig pub *17*, 547-560

Cohen, S S, 1950, Studies on commercial typhus vaccines IV The chemical composition of the antigens of commercial typhus vaccine J Immunol *65*, 475-483

Cohen, S S, and Chargaff, E, 1944, Studies on the composition of *Rickettsia prowazeki*, J Biol Chem *154*, 691-704

Cole, M M, and Burden, G S, 1956, Phosphorus compounds as ovicides and adulticides against body lice, J Econ Entomol *49*, 747-50

Cox, H R, 1938, Use of yolk sac of developing chick embryo as medium for growing rickettsiae of Rocky Mountain spotted fever and typhus groups, Pub Health Rep *53*, 2241-2247

———, 1941, Cultivation of rickettsiae of the Rocky Mountain spotted fever, typhus, and Q fever groups in the embryonic tissues of developing chicks, Science *94*, 399-403

———, 1948, Method for the preparation and standardization of rickettsial vaccines Symposium on Rickettsial Diseases, Dec 1946, Boston, American Association for the Advancement of Science

Craigie, J, 1945, Application and control of ethyl-ether-water interface effects to the separation of rickettsiae from yolk sac suspensions, Canad J Res, E, *23*, 104-114.

Craigie, J, Watson, D W, Clark, [redacted] M, and Malcomson, M E, 1946, The serological relationships of the rickettsiae of epidemic and murine typhus, Canad J Res, E, *24*, 84-103

Davis, D E, 1947, The use of DDT to control murine typhus fever in San Antonio, Texas, Pub Health Rep *62*, 449-463.

Ding, E., 1943, Über die Schutzwirkung verschiedener Fleckfieberimpfstoffe beim Menschen und den Fleckfieberverlauf nach Schutzimpfung Ztschr Hyg u Infektionskr *124*, 670-682

Durand, P, and Giroud, P, 1940, Essais de vaccination contre le typhus historique au moyen de rickettsias tuées par le formol (*souches pulmonaires*), Compt. rend Acad sc *210*, 493-496

Dyer, R. [redacted], Rumreich, A, and Badger, L F, 1931, Typhus fever. A virus of the typhus type derived from fleas collected from wild rats Pub Health Rep *46*, 334-338

Ecke, R S, Gilliam, A G, Snyder, J C, Yeomans, A, Zarafonetis, C J, and Murray, E S, 1945, The effect of Cox-type vaccine on louse-borne typhus fever, Am J Trop Med *25*, 447-462

Eddy, G W, Cole, M M, Couch, M D, and Selhime, A, 1955, Resistance of human body lice to insecticides, Pub Health Rep *70*, 1035-1038

van den Ende, M, Stuart-Harris, C. H, Fulton, F., Niven, J [redacted] F, Andrewes, C H, Begg, A M, Elford, W J, White, M H G, Hawley, W L, Mills, K C, Hamilton, F, and Thomas, C C, 1946, Chemotherapeutic and other studies of typhus, Series Medical Research Council Special Report, No 255, London, His Majesty's Stat. Off

Epstein, H, 1922, Beitrage zur Kenntnis der *Rickettsia prowazeki*, Zentralbl Bakt *87*, 553-556

Fox, J P, Jordan, M E, and Gelfand, H M, 1957, Immunization of man against epidemic typhus by infection with avirulent *Rickettsia prowazeki* strain E IV Persistence of immunity and a note as to differing complement-fixation antigen requirements in post-infection and post-vaccination sera J Immunol *79*, 348-54.

Fox, J P, Montoya, J A, Jordan, M E, and Espinosa, M M, 1955, Immunization of man against epidemic typhus by infection with avirulent *Rickettsia prowazeki* (strain E) III The serologic response and occurrence of post-vaccination reactions in groups vaccinated under field conditions in Peru Am J Hyg *61*, 183-196

Fracastorius, H, 1546, De Contagione et Contagiosis Morbis et Eorum Curatione, Libri III [Translated by W C Wright, New York, Putnam, 1930]

Fuller, H S, 1953, Studies of human body lice, *Pediculus humanus corporis* II Quantitative comparisons of the susceptibility of human body lice and cotton rats to experimental infection with epidemic typhus rickettsiae Am J Hyg *58*, 188-206

———, 1954, Quantitative comparisons of responses in cotton rats and albino rats and albino mice to measured doses of epidemic typhus rickettsiae, J Immunol *73*, 138-45

Fulton, F, and Begg, A M, 1946, Chemotherapeutic and other studies of typhus V The antigenic structure of typhus rickettsiae Medical Research Council, Special Report Series No 255, pp 163-191, London, His Majesty's Stat Off

Gerhard, W W, 1837, On the typhus fever which occurred at Philadelphia in the spring and summer of 1836, etc Am J M Sc. *19*, 289-322

Gildemeister, E, and Haagen, E, 1940, Fleckfieberstudien I Mitteilung: Nachweis eines Toxins in Rickettsien—Eikulturen (*Rickettsiae mooseri*) Deutsche med Wchnschr *66*, 878-880

Gilliam, A G, 1946, Efficacy of Cox-type vaccine in the prevention of naturally acquired louse-borne typhus fever, Am J. Hyg *44*, 401-410

Giroud, P, 1938, Essai de mise en évidence des anticorps du typhus exanthématique par un test cutané, Compt rend Soc biol *127*, 397-399

Giroud, P, Boyer, J, and Vargues, R, 1950, A propos de quelques cas de typhus exanthématique survenus chez des sujets non parasités, Presse méd *58*, 334-336

Goldberger, J, and Anderson, J F, 1912, The transmission of typhus fever, with especial reference to transmission by the head louse (*Pediculus capitis*), Pub Health Rep *27*, 297-307

Goldwasser, R A, and Shepard, C C, 1958, Staining of complement and modifications of fluorescent antibody procedures, J Immunol *80*, 122-31

Greeley, D, 1948, Methods of application of DDT, Symposium on Rickettsial Diseases Dec 1946, Boston, American Association for the Advancement of Science

Haddon, W, Jr, 1956a, An artificial membrane and apparatus for the feeding of the human body louse *Pediculus humanus corporis*, Am J Trop Med *5*, 315-25

———, 1956b, The maintenance of the human body louse Pediculus humanus corporis through complete cycles of growth by serial feeding through artificial membranes, Am J Trop Med *5*, 326-30

Hamilton, H L 1945, Specificity of the toxic factors associated with the epidemic and the murine strains of typhus rickettsiae, Am J Trop Med *25*, 391-395

Hawksley, J C, and Stokes [illegible] J, 1950 A case of Brill's disease in London, Lancet *2*, 97-99

Hayes, W J, and Gaines, T B, 1950, Control of Norway rats with residual rodenticide warfarin, Pub Health Rep *65*, 1537-1555

Hone, F S, 1922, A series of cases closely resembling typhus fever, M J Australia *1*, 1-13

Hopps, H E, Hahn, F E, Wisseman, C L, Jr, Jackson, [illegible] B, and Smadel, J E, 1956, Metabolic studies of rickettsiae III Studies of transamination, oxidative phosphorylation and glutamate-2-C^{14} incorporation by purified suspensions of *Rickettsia mooseri* J Bact *71*, 708-16

Hurlbut, H S, Peffly, R L, and Salah, A A, 1954, DDT resistance in Egyptian body lice, Am J Trop Med *3*, 922-9

Kostrzewski, J, Gruzewski, A, and Milewska, L, 1957, Further remarks on the possible anticipation of recrudescences of typhus, Przeglad Epidem *11*, 21-26 From T [illegible] Bulletin 1957, *54*, 801

Laigret, J, and Durand P, 1939, La vaccination contre le typhus exanthématique Nouvelle technique de préparation du vaccin emploi des cerveaux de souris Bull Acad méd *122*, [illegible] 89

Lee, R I, 1913, Typhus fever (Brill's disease) at the Massachusetts General Hospital in ten years (Oct 1, 1902, to Oct 1, 1912) Boston Med & Surg J *168*, 122-127

Ley, H R, and Smadel, J E, 1954, Antibiotic therapy of rickettsial diseases, Antibiotics & Chemother *4*, 792-802

Lim, C E, and Kurotchkin, T J, 1929, A precipitin test for typhus fever with specific substance of *Bacillus proteus* X 19, Nat Med J China *15*, 6-10

Loeffler, W, and Mooser, H, 1952, Ein weiterer Fall von Brill-Zinsserscher Krankheit in Zurich, Spater Ruckfall bei klassischem Fleckfieber, Schweiz med Wchnschr *82*, 493

MacArthur, W P, 1954, The athenian plague a medical note, Classical Quarterly *4* (n s), Nos 3-4, 171-174

Macchiavello, A, 1937, Estudios sobre tifus exantematico III Un nuevo método para teñir Rickettsia Rev chilena hig *1*, 101-106

McCrae, T, 1907 Typhus fever *in* Osler, Modern Medicine, vol 2, [illegible] 232, Philadelphia, Lea & Febiger

Maxcy, K F, 1926, Clinical observations on endemic typhus (Brill's disease) [illegible] the United States, Pub Health Rep *41*, 1213-1220.

———, 1929, Typhus fever in the United States, Pub Health Rep *44*, 1735-1742

Miller, [illegible] S, and Beeson, P B, 1946, Murine typhus fever, Medicine *25*, 1-15.

Monteiro, J L, 1931, Estudos sobre [illegible] typho exantematico de São Paulo, Mem Inst Butantan *6*, 1-135

Mooser, H, 1928, Experiments relating to the pathology and the etiology of Mexican typhus (tabardillo), J Infect Dis *43*, 241-272

———, 1932, Essai sur l'histoire naturelle du typhus exanthematique, Arch Inst Pasteur de Tunis *21*, 1-19

———, 1942, Le typhus exanthematique Cours de méd et chir de guerre, Genève, sept, Q [illegible] du I er corps d'armee [Quoted from *Mooser*, 1946]

———, 1945, Die Beziehungen des murinen Fleckfiebers zum klassischen Fleckfieber, Acta Tropica (Suppl) 4, Basel Reinhardt

——— 1946 Twenty years of research in typhus fever, Schweiz med Wchnschr 76, 877-882

Mooser, H, Castaneda, M R, and Zinsser, H, 1931a Rats as carriers of Mexican typhus fever, J A M A *97*, 231-232

———, 1931b, The transmission of Mexican typhus from rat to rat by *Polyplax spinulosus*, J Exper Med *54*, 567-575

Mooser, H, and Loeffler, W, 1946, Ein Fall Sogenannter Brillscher Krankheit in Zurich, Schweiz med Wchnschr *76*, 150-153

Morgan, H R, Neva, F A, Fahey, R J, and Finland, M, 1948, Report of two serologically proved cases of typhus fever in Irish-born residents of Boston, New England J Med *238*, 871-873

Murchison, C, 1884, A Treatise on the Continued Fevers of Great Britain, ed 3, London, Longmans, Green

Murray, E S, Baehr, G, Shwartzman, G, Mandelbaum, R A, Rosenthal, N, Doane, J C, Weiss, L B, Cohen S, and Snyder, J C, 1950, Brill's disease I Clinical and laboratory diagnosis J A M A *142*, 1059-1066

Murray, E S, Cohen, S, Jampol, J, Ofstrock, A, and Snyder, J C, 1952, Epidemic typhus antibodies in human subjects in Boston, Mass, New England J Med *246*, 355-359

Murray, E S, and Gaon, J, 1958 Personal Communication

Murray, E S, Ofstrock, A, and Snyder, J C, 1952, Antibody response of human subjects to epidemic typhus vaccine three to eight years after previous immunization, J Immunol *68*, 207-218

Murray, E S, Pšorn, T, Djakovic, P, Sielski, S., Broz, V, Ljupša, F, Gaon, J, Pavlevic, R, and Snyder, J C, 1951, Brill's Disease IV Study of 26 cases in Yugoslavia Am J Pub Health *41*, 1359-1369

Murray, E S, and Snyder, J C, 1951, Brill's disease II Etiology Am J Hyg *53*, 22-32

———, 1953, Brill-Zinsser Disease The interepidemic reservoir of epidemic louse-borne typhus fever Presented at VI International Congress of Microbiology, Rome, Italy

National Research Council, Division of Medical Sciences, Committee on Pathology, 1953 Pathology of epidemic typhus II Report of fatal cases studied by the U S A Typhus Commission in Cairo, Egypt, during 1943-45 Arch Path *56*, 397-435, 512-53

Neill, M H, 1917, Experimental typhus fever in guinea pigs A description of a scrotal lesion in guinea pigs infected with Mexican typhus Pub Health Rep *32*, 1105-1108

Neva, F A, and Snyder, J C, 1952, Susceptibility of the white rat, with note on pathologic changes, J Infect Dis *91*, 72-78

———, 1955, Studies on the toxicity of typhus rickettsiae III Observations on the mechanism of toxic death in white mice and white rats J Infect Dis *97*, 73-87

Nicolle, C, Comte, C, and Conseil, E, 1909, Transmission expérimentale du typhus exanthématique par le pou du corps, Compt rend Acad sc *149*, 486-489

Nicolle, C, Conseil, E, and Conor, A, 1911, Le typhus experimentale du cobaye, Compt rend Acad sc *152*, 1632-1634

Otto, R, and Munter, H, 1930, Fleckfieber *in* Kolle, Kraus, and Uhlenhuth, Handbuch der pathogenen Mikroorganismen, ed 3, vol 8, pp 1107-1262, Jena, Fischer

Palacios, R, Chavez, F, and Avendano, O, 1935, *Aislamiento de virus murino de individuos inoculados* con vacun Blanc, Rev Inst bact de Chile *4*, 49-52

Paterson, P Y, Wisseman, C L, Jr, and Smadel, J E, 1954, Studies of rickettsial toxins I Role of hemolysis in fatal toxemia of rabbits and rats J. Immunol *72*, 12-23

Paullin, J. E, 1913, Typhus fever with a report of cases, South M J *6*, 36-43

Philip, C B, 1948, The reservoirs of infection in rickettsial diseases Symposium on Rickettsial Dis-

serological response to infection with murine typhus by previous immunization with epidemic typhus vaccine, Proc. Soc Exper. Biol & Med *59*, 248-251

Pollard, M, Wilson, D J, Livesay, H R, and Woodland, J C, 1946, The oral transmission of murine typhus strains in humans, Texas Rep Biol & Med *4*, 446-451

Price, W H, 1955, Studies on the interepidemic survival of louse-borne epidemic typhus fever, J Bact *69*, 106-107

Price, W H, Emerson, H, Nagel, H, Blumberg, R, and Talmadge, S, 1958, Ecological studies on the interepidemic survival of louse-borne epidemic typhus fever, Am J Hyg *67*, 154-178

von Prowazek, S, 1914, Ätiologische Untersuchungen uber den Flecktyphus in Serbien 1913 und Hamburg 1914, Beitr Klin der Infektionskr *4*, 5-31

Ricketts, H T, and Wilder, R. M, 1910a, The transmission of the typhus fever of Mexico (tabardillo) by means of the louse (*Pediculus vestamenti*), J.A.M.A *54*, 1304-1307

———, 1910b, The etiology of the typhus fever (tabardillo) of Mexico City A further preliminary report J.A.M.A *54*, 1373-1375

Ris, H, and Fox, J P, 1949, The cytology of rickettsiae, J Exper Med *89*, 681-686

da Rocha-Lima, H, 1916, Zur Aetiologie des Fleckfiebers Kriegspathol Tagung, April 26, 1916, pp 45-50, Beiheft zu Band *27*, Zentralbl allg Path

———, 1918, Schutzimpfungsversuche gegen Fleckfieber, Munch med Wchnschr *65*, 1454-1456

Sadusk, J F, Jr, 1947, Typhus fever in the United States Army following immunization Incidence, severity of the disease, modification of the clinical course and serologic diagnosis J.A.M.A *133*, 1192-1199

Sadusk, J F, Jr, and Kuhlenbeck, H, 1946, Dangers associated with the use of living "attenuated" typhus vaccine, Am J Pub Health *36*, 1027-1030

Schaechter, M, Tousimis, A J, Cohn, Z A, Rosen, H, Campbell, J, and Hahn, F E, 1957, Morphological, chemical and serological studies of the cell walls of *Rickettsia mooseri*, J Bact *74*, 822-29

Scoville, A. B, Jr, Bennett, B L, Wertman, K, and Gauld, R, 1948, The serological pattern in typhus fever II Murine Am J. Hyg *47*, 166-176

Silva-Goytia, R, 1944, Fijacion de complemento con sueros de enfermos de tifo exantemático Rev Inst salub y enferm trop *5*, 241-245

Sinclair, C G, and Maxcy, K. F, 1925, Mild typhus (Brill's disease) in the lower Rio Grande Valley, Pub Health Rep *40*, 241-248

Smadel, J. E, 1948, The complement-fixation and agglutination reaction in rickettsial diseases Symposium on Rickettsial Diseases, Dec 1946, Boston, American Association for the Advancement of Science

Snyder, J C, 1947, Typhus fever in the second World War, California Med *66*, 3-10

———, 1948, The treatment of the rickettsial diseases of man Symposium on Rickettsial Diseases, Dec 1946, Boston, American Association for the Advancement of Science

Snyder, J C, Bovarnick, M. R., Miller, J C, and Chang, R S, 1954, Observations on the hemolytic

properties of typhus rickettsiae, J Bact 67, 724-30

Snyder, J C, Yeomans, A, Clement, D H, Murray, E S, Zarafonetis, C J D, and Turney, A, 1947, Further observations on the treatment of typhus fever with para-aminobenzoic acid, Ann Int Med 27, 1-27

Soares, J A, 1950, Doença do Brill A Proposito de Tres Casos de Recrudescencia de Tifo Exantematico Historico Observados em Braga, Bol Inst Sup d Hyg Duot Ric Jor 5, 1-18

Soper, F L, Davis, W A, Markham, F S, Riehl, L A, and Buck, P, 1945, Louse powder studies in North Africa (1943), Arch Inst Pasteur d'Algerie 23, 183-223

Strong, R P, Shattuck, G C, Sellards, A W, Zinsser, H, and Hopkins, J G, 1920, Typhus Fever with Particular Reference to the Serbian Epidemic, Cambridge, Harvard Univ Press

Topping, N H, Bengtson, I A, Henderson, R G, Shepard, C C, and Shear, M J, 1945, Studies of typhus fever National Institute of Health Bull No 183, U S Govt Printing Office, Washington, D C

Varela, G, and Zozaya, J, 1944, Distribucion en México de las cepas de tifo exantematico hasta ahora estudias, Rev Inst salub y enferm trop 6, 11-14

Wattenberg, L W, Elisberg, B L Wisseman, C L, Jr, and Smadel, J E, 1955, Studies of rickettsial toxins II Altered vascular physiology in rickettsial toxemia of mice J Immunol 74, 147-57

Weigl, R, 1920, Untersuchungen und Experimente an Fleckfieberlausen Die Technik der Rickettsia-Forschung Beitr Klin der Infektionskr 8, 353-376

———, 1930, Die Methoden der aktiven Fleckfieber-Immunisierung Bull Internat Acad Polonaise sci et lettres (Cl Med), July 1930, 25-62

Weil, E, and Felix, A 1916, Zur serologischen Diagnose des Fleckfiebers, Wien klin Wchnschr 29, 33-35

Wheatland, F T, 1926, A fever resembling a mild form of typhus fever, M J Australia 1, 261-266

Wheeler, C M, 1943, Report to the Typhus Conference, London, Nov 1943 Files of the National Research Council, Washington, D C

Wisseman, C L, Jr, Hahn, F E, Jackson, E B, and Smadel, J E, 1951, Metabolic studies of rickettsiae pathway of glutamate oxidation in suspensions of Rickettsia mooseri, Fed Proc 10 424

Wolbach, S B, 1940, The rickettsial diseases A general survey in Virus and Rickettsial Diseases, pp 789-816, Cambridge, Harvard Univ Press

———, 1948, The pathology of typhus fever Symposium on Rickettsial Diseases, Dec 1946, Boston, American Association for the Advancement of Science

Wolbach, S B, and Todd, J L, 1920, Note sur l'étiologie et l'anatomie pathologique du typhus exanthematique au Mexique, Ann Inst Pasteur 34, 153-158

Wolbach, S B, Todd, J L, and Palfrey, F W, 1922, The Etiology and Pathology of Typhus, Cambridge, Harvard Univ Press

Woodward, T E, 1948, Clinical course of murine typhus Symposium on Rickettsial Diseases, Dec 1946, Boston, American Association for the Advancement of Science

Woodward, T E, and Bland, E F, 1944, Clinical observations in typhus fever with special reference to the cardiovascular system, J A M A 126, 287-293

Worms, R, 1950, Le problème du typhus sporadique, Semaine hôp Paris 26, 1-15

Wright, J W, and Brown, A W A, 1957, Survey of possible insecticide resistance in body lice, Bull World Health Organ 16, 9-31

Yeomans, A, 1947, Typhus fever in Oxford Medicine, vol 5, pp 439-466 (23), New York, Oxford Univ Press

Yeomans, A, Snyder, J C, Murray, E S, Ecke, R S, and Zarafonetis, C J D, 1945, Azotemia in typhus fever, Ann Int Med 23, 711-753

Zarafonetis, C J D, Ecke, R S, Yeomans, A, Murray, E S, and Snyder, J C, 1946, Serologic studies in typhus-vaccinated individuals III Weil Felix and complement-fixation findings in epidemic typhus fever occurring in the vaccinated J Immunol 53, 15-30

Zinsser, H, 1934, Varieties of typhus virus and the epidemiology of the American form of European typhus fever (Brill's disease), Am J Hyg 20, 513-532

———, 1935, Rats, Lice, and History, Boston, Little

———, 1937, The rickettsia diseases Varieties, epidemiology, and geographical distribution Am J Hyg 25, 430-463

———, 1940, Epidemiology and immunity in the rickettsial diseases in Virus and Rickettsial Diseases, pp 872-907, Harvard School of Public Health Cambridge, Harvard Univ Press

Zinsser, H, and Castaneda, M R, 1932, Studies on typhus fever IX On the serum reactions of Mexican and European typhus rickettsia J Exper Med 56, 455-467

Zinsser, H, Wei, H, and FitzPatrick, F, 1938, Further studies of agar-slant tissue cultures of typhus rickettsiae, Proc Soc Exper Biol & Med 38, 285-288

Murray, E. S, and Gaon, J, 1958 Personal Communication

Murray, E S, *Ofstrock, A, and Snyder, J C, 1952,* Antibody response of human subjects to epidemic typhus vaccine three to eight years after previous immunization, J Immunol *68, 207-218*

Murray, E S, Pšorn, T, Djakovic, P, Sielski, S, Broz, V, Ljupša, F, Gaon, J, Pavlevic, R, and Snyder, J C, 1951, Brill's Disease IV Study of 26 cases in Yugoslavia Am J Pub Health *41,* 1359-1369

Murray, E S, and Snyder, J C, 1951, Brill's disease II Etiology Am J Hyg *53, 22-32*

———, 1953, Brill-Zinsser Disease The interepidemic reservoir of epidemic louse-borne typhus fever. Presented at VI International Congress of Microbiology, Rome, Italy

National Research Council, Division of Medical Sciences, Committee on Pathology, 1953 Pathology of epidemic typhus II Report of fatal cases studied by the U S A Typhus Commission in Cairo, Egypt, *during 1943-45 Arch Path 56, 397-435,* 512-53

Neill, M H, 1917, Experimental typhus fever in guinea pigs A description of a scrotal lesion in guinea pigs infected with Mexican typhus Pub Health Rep *32,* 1105-1108

Neva, F A, and Snyder, J C, 1952, Susceptibility of the white rat, with note on pathologic changes, J Infect Dis *91,* 72-78

———, 1955, Studies on the toxicity of typhus rickettsiae III Observations on the mechanism of toxic death in white mice and white rats J Infect Dis *97,* 73-87.

Nicolle, C, Comte, C., and Conseil, E, 1909, Transmission expérimentale du typhus exanthematique par le pou du corps, Compt rend Acad sc *149,* 486-489

Nicolle, C, Conseil, E, and Conor, A, 1911, Le typhus expérimentale du cobaye, Compt rend Acad *sc 152, 1632-1634*

Otto, R, and Munter, H, 1930, Fleckfieber *in* Kolle, Kraus, and Uhlenhuth, Handbuch der pathogenen Mikroorganismen, ed 3, vol 8, pp 1107-1262, Jena, Fischer

Palacios, R, Chavez, F, and Avendano, O, 1935, Aislamiento de virus murino de individuos inoculados con vacun Blanc, Rev Inst bact de Chile *4,* 49-52

Paterson, P Y, Wisseman, C L, Jr, and Smadel, *J E, 1954, Studies of rickettsial toxins I Role* of hemolysis in fatal toxemia of rabbits and rats J Immunol *72,* 12-23

Paullin, J. E, 1913, Typhus fever with a report of cases, South M J *6,* 36-43

Philip, C B, 1948, *The* reservoirs of infection in [illegible]

[illegible] J, and Gauld, R, *1948,* [illegible] phus fever I. Epidemic Am J Hyg *47,* 150-165

Plotz, H, and Wertman, K, 1945, Modification of serological response to infection with murine typhus by previous immunization with epidemic typhus vaccine, Proc. Soc Exper. Biol & Med *59,* 248-251

Pollard, M, Wilson, D J, Livesay, H R, and Woodland, J C, 1946, The oral transmission of murine typhus strains in humans, Texas Rep Biol & Med *4,* 446-451

Price, W H, 1955, Studies on the interepidemic survival of louse-borne epidemic typhus fever, J Bact *69,* 106-107

Price, W. H, Emerson, H., Nagel, H, Blumberg, R, and Talmadge, S, 1958, Ecological studies on the interepidemic survival of louse-borne epidemic typhus fever, Am J Hyg *67,* 154-178

von Prowazek, S, 1914, Ätiologische Untersuchungen über den Flecktyphus in Serbien 1913 und Hamburg 1914, Beitr. Klin der Infektionskr *4,* 5-31

Ricketts, H T, and Wilder, R M, 1910a, The transmission of the typhus fever of Mexico (tabardillo) by means of the louse (*Pediculus vestamenti*), J A M A *54,* 1304-1307

———, 1910b, The etiology of the typhus fever (tabardillo) of Mexico City A further preliminary report J A M A *54,* 1373-1375.

Ris, H, and Fox, J P, 1949, The cytology of rickettsiae, J Exper Med *89,* 681-686

da Rocha-Lima, H, 1916, Zur Aetiologie des Fleckfiebers Kriegspathol Tagung, April 26, 1916; pp 45-50, Beiheft zu Band *27,* Zentralbl allg Path

———, 1918, Schutzimpfungsversuche gegen Fleckfieber, Munch med Wchnschr *65,* 1454-1456

Sadusk, J F, Jr, 1947, Typhus fever in the United States Army following immunization Incidence, severity of the disease, modification of the clinical course and serologic diagnosis J A M A *133,* 1192-1199

Sadusk, J F, Jr, and Kuhlenbeck, H, 1946, Dangers associated with the use of living "attenuated" typhus vaccine, Am J Pub Health *36,* 1027-1030

Schaechter, M, Tousimis, A J, Cohn, Z A, Rosen, H, Campbell, J, and Hahn, F E, 1957, *Morphological, chemical and serological studies of the* cell walls of *Rickettsia mooseri,* J Bact *74,* 822-29

Scoville, A B, Jr, Bennett, B L, Wertman, K, and Gauld, R, 1948, The serological pattern in *typhus fever. II Murine Am J Hyg 47, 166-176*

Silva-Goytia, R, 1944, Fijación de complemento con sueros de enfermos de tifo exantemático Rev Inst salub y enferm trop *5,* 241-245

Sinclair, C G, and Maxcy, K F, 1925, Mild typhus *(Brill's disease) in the lower Rio Grande* Valley, Pub Health Rep *40,* 241-248

Smadel, J E, 1948, The complement-fixation and agglutination reaction in rickettsial diseases Symposium on Rickettsial Diseases, Dec 1946, Boston, *American Association for the Advancement of Science*

Snyder, J C, 1947, Typhus fever in the second World War, California Med *66,* 3-10

———, 1948, The treatment of the rickettsial diseases *of man Symposium on Rickettsial Diseases,* Dec 1946, Boston, American Association for the Advancement of Science

Snyder, J. C, Bovarnick, M R, Miller, J C, and Chang, R. S, 1954, Observations on the hemolytic

properties of typhus rickettsiae J Bact 67, 724-30

Snyder, J C, Yeomans, A, Clement, D H, Murray, E S, Zarafonetis, C J D, and Tierney, A, 1947, Further observations on the treatment of typhus fever with para-aminobenzoic acid Ann Int Med 27, 1-27

Soares, J A, 1950, Doença do Brill A Proposito de Tres Casos de Recrudescencia de Tifo Exantematico Historico Observados em Braga, Bol Inst Sup d Hyg Dout Ric Jor 5, 1-18

Soper, F L, Davis, W A, Markham, F S, Riehl, L A, and Buck, P, 1945, Louse powder studies in North Africa (1943), Arch Inst Pasteur d'Algerie 23, 183-223

Strong, R P, Shattuck, G C, Sellards, A W, Zinsser, H, and Hopkins, J G 1920, Typhus Fever with Particular Reference to the Serbian Epidemic, Cambridge, Harvard Univ Press

Topping, N H, Bengtson, I A Henderson, R G, Shepard, C C, and Shear, M J, 1945 Studies of typhus fever National Institute of Health Bull No 183, U S Govt Printing Office Washington, D C

Varela, G, and Zozaya, J, 1945, Distribucion en Mexico de las cepas de tifo exantematico hasta ahora estudias, Rev Inst salub y enferm trop 6, 11-14

Wattenberg, L W, Elisberg B L, Wisseman, C L, Jr, and Smadel, J E, 1955, Studies of rickettsial toxins II Altered vascular physiology in rickettsial toxemia of mice J Immunol 74, 147-57

Weigl, R, 1920, Untersuchungen und Experimente an Fleckfieberläusen Die Technik der Rikettsia-Forschung Beitr Klin der Infektionskr 8, 353-376

———, 1930, Die Methoden der aktiven Fleckfieber-Immunisierung Bull Internat Acad Polonaise sci et lettres (Cl Med), July 1930, 25-62

Weil, E, and Felix A, 1916, Zur serologischen Diagnose des Fleckfiebers, Wien klin Wchnschr 29, 33-35

Wheatland, F T, 1926, A fever resembling a mild form of typhus fever, M J Australia 1, 261-266

Wheeler, C M, 1943, Report to the Typhus Conference, London, Nov 1943 Files of the National Research Council, Washington D C

Wisseman, C L, Jr, Hahn, F E, Jackson, E B, and Smadel, J E 1951 Metabolic studies of rickettsiae pathway of glutamate oxidation in suspensions of Rickettsia mooseri, Fed Proc 10, 424

Wolbach, S B, 1940, The rickettsial diseases A general survey in Virus and Rickettsial Diseases, pp 789-816, Cambridge, Harvard Univ Press

———, 1948, The pathology of typhus fever Symposium on Rickettsial Diseases, Dec 1946, Boston, American Association for the Advancement of Science

Wolbach, S B, and Todd, J L, 1920, Note sur l'etiologie et l'anatomie pathologique du typhus exanthematique au Mexique, Ann Inst Pasteur 34, 153-158

Wolbach, S B, Todd, J L, and Palfrey, F W, 1922, The Etiology and Pathology of Typhus, Cambridge, Harvard Univ Press

Woodward, T E, 1948, Clinical course of murine typhus Symposium on Rickettsial Diseases, Dec 1946, Boston, American Association for the Advancement of Science

Woodward, T E, and Bland, E F, 1944, Clinical observations in typhus fever with special reference to the cardiovascular system, J A M A 126, 287-293

Worms, R, 1950, Le probleme du typhus sporadique, Semaine hôp Paris 26, 1-13

Wright, J W and Brown, A W A, 1957, Survey of possible insecticide resistance in body lice, Bull World Health Organ 16, 9-31

Yeomans, A, 1947, Typhus fever in Oxford Medicine, vol 5, pp 439-466 (23), New York, Oxford Univ Press

Yeomans, A, Snyder, J C, Murray, E S, Ecke, R S, and Zarafonetis, C J D, 1945, Azotemia in typhus fever, Ann Int Med 23, 711-753

Zarafonetis, C J D, Ecke, R S, Yeomans, A, Murray, E S, and Snyder, J C, 1946, Serologic studies in typhus-vaccinated individuals III Weil-Felix and complement-fixation findings in epidemic typhus fever occurring in the vaccinated J Immunol 53, 15-30

Zinsser, H, 1934, Varieties of typhus virus and the epidemiology of the American form of European typhus fever (Brill's disease), Am J Hyg 20, 513-532

———, 1935, Rats, Lice, and History, Boston, Little

———, 1937, The rickettsia diseases Varieties, epidemiology, and geographical distribution Am J Hyg 25, 430-463

———, 1940, Epidemiology and immunity in the rickettsial diseases in Virus and Rickettsial Diseases, pp 872-907, Harvard School of Public Health Cambridge, Harvard Univ Press

Zinsser, H, and Castaneda, M R, 1932, Studies on typhus fever IX On the serum reactions of Mexican and European typhus rickettsia J Exper Med 56, 455-467

Zinsser, H, Wei, H, and FitzPatrick, F, 1938, Further studies of agar-slant tissue cultures of typhus rickettsiae, Proc Soc Exper Biol & Med 38, 285-289

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43

The Spotted-Fever Group

The spotted-fever group includes, in addition to Rocky Mountain spotted fever of the United States and Canada, apparently identical infections in Mexico (*Fiebre manchada, fiebre de choix* and *fiebre pinta*), Panama, Colombia (Tobiã fever), and Brazil (São Paulo and Minas Gerais fevers), as well as other tick- or mite-borne diseases such as tick-borne typhus (boutonneuse fever) of the Mediterranean regions of Europe and Africa, and the Crimea; identical or closely related tick-borne infections (tick-borne typhus, tick typhus or tick-bite fever) of all parts of Africa; Queensland tick typhus, rickettsialpox, maculatum disease, and the tick-borne rickettsioses of India (Indian tick typhus) and the Soviet Union (Siberian tick typhus)

ROCKY MOUNTAIN SPOTTED FEVER

(SYNONYMS Mountain fever, typhomalaria fever, Bull fever, black fever, blue disease, spotted fever and American spotted fever The author suggests "New World spotted fever")

INTRODUCTION

Rocky Mountain spotted fever is an acute, endemic, infectious disease which is recognized as being one of the severest of all infections (Rosenblum et al., 1952). Essentially, it is a generalized intracellular infection of the small peripheral blood vessels The causative agents are *Rickettsia rickettsii* (Bengtson, 1948), *Dermacentroxenus rickettsi*, Wolbach, 1919, *Rickettsia rickettsi* (Brumpt, 1927), *Rickettsia brasiliensis* (Monteiro, 1931), and *Rickettsia typhi* (do Amaral and Monteiro, 1932). The only known method of natural transmission to animals or man is through the medium of infected ticks

HISTORY

Rocky Mountain spotted fever was first reported from the Rocky Mountain region of Montana in 1873. In 1896, Wood gave a detailed account of the disease in Idaho This was followed by Maxey's report in 1899 which described the disease as "an acute, endemic, non-contagious but probably infectious, febrile disease, characterized clinically by a continuous moderately high fever, severe arthritic and muscular pains, and a profuse petechial or purpural eruption in the skin, appearing first on the ankles, wrists and forehead, but rapidly spreading to all parts of the body."

Wilson and Chowning, 1902, investigated the disease and claimed an erythrocytic parasite, *Piroplasma hominis*, was the causative agent. They also suggested that the disease was caused by the bite of the wood tick, *Dermacentor andersoni* In 1906a, Ricketts achieved the first transmission of the disease to experimental animals (guinea pigs and monkeys) by inoculation with infected human blood.

In the same year, Ricketts, 1906b, and King, 1906, independently reported that the

disease was transmitted by the wood tick,* and in the following year, Ricketts, *1907b*, demonstrated the occurrence of naturally infected ticks in the *Bitter Root Valley* of western Montana. In 1907c, Ricketts showed that the infectious agent, acquired by immature ticks at any stage, was carried through to the adults, and that at least a certain proportion of infected females transmitted the infection to their progeny through eggs. In 1909, Ricketts described the disease-producing micro-organism in smears prepared from the blood of man, monkey, guinea pig and from tissues of the wood tick. In 1910, Ricketts and Wilder, by cross-immunity experiments, showed that Rocky Mountain spotted fever and typhus fever are distinct and separate entities. In 1919, Wolbach reported the results of his careful etiologic and pathologic studies and named the etiologic organisms *Dermacentroxenus rickettsi*. Wolbach differentiated between this organism and nonpathogenic organisms in tick tissues and was the first to demonstrate the intranuclear multiplication of the rickettsiae in tick tissue. In *1923*, Parker reported the stage-to-stage transmission of the spotted fever rickettsiae in the life-cycle of both *Dermacentor andersoni* and *Haemaphysalis leporis-palustris*. In 1931 Rumreich et al recorded the occurrence of spotted fever in the eastern United States and in the same year Dyer and co-workers (1931) reported on the transmission of the infection by the dog tick *Dermacentor variabilis*.

Rocky Mountain spotted fever was first recognized in Brazil by Piza et al (1931), in Colombia by Patino et al (1937), in Canada by Hearle (*1938*) and Gibbons (1942), in Mexico by Bustamente and Varela (*1943*), and in Panama by De Rodaniche and Rodaniche (1950).

Clinical Picture

Rocky Mountain spotted fever is recognized as being one of the most severe of all infectious diseases. In many of its aspects, Rocky Mountain spotted fever resembles typhus, the chief differences being the duration of fever, the severity of the disease, and the time of appearance and the location of the rash. Attacks range from mild ambulatory and abortive forms to rapidly terminating fatal infections. The fatality rate varies in different regions and for different ages. In vaccinated persons and young children, the attacks are frequently mild and atypical.

In nonvaccinated adults, the incubation period of the disease is usually 4 to 8 days but may show extremes of 2 to 12 days. The prodromal manifestations are listlessness, malaise, headache, loss of appetite and chilling sensations. Onset of the disease is usually abrupt, coming as a rule in the late afternoon or early evening. It is manifested by a definite chill, pronounced headache, severe aches and pains in the muscles, bones and joints, profound prostration and a rapidly rising fever that continues to mount into the second week. Myalgia and arthralgia are marked, and in the more severe form, epistaxis may occur early.

As the disease progresses, mental confusion, restlessness, dulling of the senses and lethargy progressing to coma may be noted. Muscular twitching, fibrillary tremors, purposeless movements and abnormal neurologic signs may occur. Indeed, the picture may be that of an acute encephalitis. Fevers of 104° to 105° F. or higher are common. Remissions of 1° to 3° F. may be observed in morning temperatures. With recovery from the acute manifestations of illness the fever falls, either by rapid or slow lysis, usually at about the end of the 3rd week, although mild cases may become afebrile before the end of the 2nd week. Early in the disease the pulse is of good volume and slow, averaging approximately 90 beats per minute. One of the characteristics of spotted fever at its onset is the early disproportion of the pulse and temperature ratio. When myocardial weakening occurs in severe cases as a result of toxemia, loss of volume and strength of the pulse occurs. As a result of myocardial involvement and vascular collapse, the blood pressure falls, and the first heart sound becomes muffled and indistinct. The pulmonary edema which then appears often indicates a fatal termination within a few hours. The respirations are at

* Doctors McCalla and Brereton of Boise, Idaho (reported by Ricketts, 1907a), probably performed the first experiments to show that wood ticks could transmit the disease to human subjects. These doctors infected 2 individuals in series by the bite of a tick which they had removed from one of their patients. These experiments were not known to Ricketts, 1906b, or King, 1906, at the time they did their work, although the studies of McCalla and Brereton preceded theirs by more than a year.

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Rocky Mountain spotted fever was first reported from the Rocky Mountain region of Montana in 1873. In 1896, Wood gave a detailed account of the disease in Idaho. This was followed by Maxey's report in 1899 which described the disease as "an acute, endemic, non-contagious but probably infectious, febrile disease, characterized clinically by a continuous moderately high fever, severe arthritic and muscular pains, and a profuse petechial or purpural eruption in the skin, appearing first on the ankles, wrists and forehead, but rapidly spreading to all parts of the body"

Wilson and Chowning, 1902, investigated the disease and claimed an erythrocytic parasite, *Piroplasma hominis*, was the causative agent They also suggested that the disease was caused by the bite of the wood tick, *Dermacentor andersoni* In 1906a, Ricketts achieved the first transmission of the disease to experimental animals (guinea pigs and monkeys) by inoculation with infected human blood

In the same year, Ricketts, 1906b, and King, 1906, independently reported that the

FIG 126. Typical rash on a patient suffering from Rocky Mountain spotted fever

ondary anemia The white cell count usually ranges between 12,000 and 15,000 per cu. mm , but may go as high as 30,000 Leukopenia, with white blood cell counts of 4,000 to 6,000, may be present at the onset of the disease, the differential count then shows a neutropenia The urine usually shows no abnormalities, although slight albuminuria may appear, apparently as the result of the fever, and then disappear with convalescence The cerebrospinal fluid may be under increased pressure, especially when generalized edema is present The protein content of the spinal fluid is usually normal An increase in mononuclear cells is often seen accompanying the encephalitis which [redacted] so common that it may be considered a part of the disease Nervous manifestations are quite common and include headache, restlessness, insomnia, confusion, disorientation, coma, convulsions, hyperactive and pathologic reflexes, cranial nerve palsies, paraplegia and hemiplegia In fulminating cases, coma usually precedes death, which occurs most often at the end of the 2nd week of illness

Rosenblum et al (1952) state that in some patients the acute changes clear up with convalescence, but that in others the residual damage persists for longer than a year and may be considered permanent Thirty-seven patients of all ages who had had spotted-fever infections from 1 to 8 years previously were re-examined by Rosenblum [redacted] al (1952) Twenty-one of the 37 patients showed some type of neurologic sequelae at the time of re-examination Fourteen showed a history of symptoms related to the central nervous system, 6 had neurologic signs, and 12 clearly showed abnormal electroencephalograms An additional 12 patients had borderline elec-

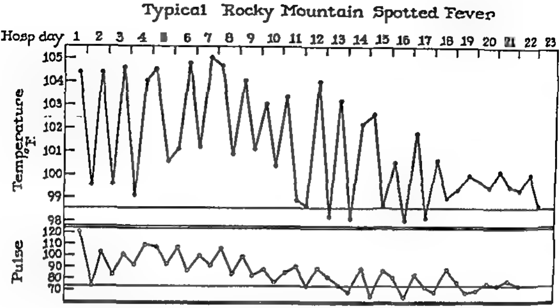

FIG 125 Temperature and pulse-rate curves of a typical case of Rocky Mountain spotted fever before the introduction of treatment by para-aminobenzoic acid and serum (Dr. R R Parker, Rocky Mountain Spotted Fever Laboratory, U. S Public Health Service, Hamilton, Mont)

first normal or but slightly increased. They accelerate, in severe cases, with alterations of the temperature and pulse ratio The increase in rates often indicates the development of pneumonia Terminal temperatures of 108° F, pulse rates of 140 to 160 and respiration rates of 40 to 60 are not uncommon (Baker, 1951). A short incubation period, high pulse rate, severe mental changes, edema, anuria or oliguria, a fine petechial rash and pneumonia are poor prognostic signs. Figure 125 shows temperature and pulse rate curves of a typical untreated case of Rocky Mountain spotted fever.

A distinctive rash usually appears on the 2nd to the 4th day of the disease but may be delayed until the 5th or 6th day. It may resemble the slight mottling seen in early measles The rash first appears on the ankles and the wrists and spreads rapidly to the legs, the arms and the chest The lesions are macular at first but later become maculopapular. Definite petechiae may form. The lesions are more pronounced on the extremities. The palms and the soles and at times even the face and the scalp become involved. Early in the course of the disease, the spots are less pronounced during the morning remissions of fever but progressively become more distinct each day until they are definitely petechial in all but the mildest types of infection (Fig 126). Extension of the rash usually becomes complete within 2 to 3 days so that the entire body is covered. The lesions of Rocky Mountain spotted fever do not disappear on pressure except during the early stages of the disease and can be accentuated by tourniquet application. In severe cases, the lesions become confluent, deep red or purplish in color and often necrotic. Masses of such areas may involve the entire body. The afflicted individual presents a most tragic picture if terminal gangrene develops with sloughing of dependent body parts. The eruption gradually fades as patients recover, with the process taking much longer, the more severe the disease There may be branny desquamation Pigmentation remains at the site of former eruptions For several months following recovery, overexposure to heat or cold often brings out temporary recurrence of the lesions. These have no significance and clear within a few minutes after the skin temperatures return to normal

The blood findings in Rocky Mountain spotted fever are not significant There is a lowered red blood cell count and hemoglobin content later in the disease resulting in a sec-

Mexico and Brazil Animals serving as hosts to *D andersoni* and believed to be responsible for maintaining the infection in nature are the tree squirrel, ground squirrel, snowshoe rabbit, jack rabbits (including the black-tailed jack rabbit), cottontail rabbit, porcupine, chipmunk, pack rat, wood rat, meadow mouse, deer mouse, weasel, marmot and dog It may be significant, as pointed out by Jellison (1945), that in the United States a close geographic relationship exists between spotted fever and a species of cottontail rabbit, *Sylvilagus nuttali* Parker (1938) likewise has emphasized his belief of the importance in nature of the rabbit-tick-rabbit host association in carrying at least low-grade strains of infection Most of the natural hosts show only inapparent infections, with no diagnostic gross lesions or distinctive febrile reactions, and seldom die Recently, Gould and Miesse (1954) reported the isolation of a strain of rickettsiae belonging to the spotted fever group from the tissues of a meadow mouse, *Microtus pennsylvanicus* (an apparently healthy male), collected during a survey (1952-1953) in a suburb of Alexandria, Va This is the first time that a spotted fever strain of rickettsiae has been definitely recovered from naturally infected animals collected within the United States This agent, which was found to be a mild strain, showed an incubation period of 2 to 8 days and a febrile phase of 5 to 9 days duration for inoculated guinea pigs but was not lethal No scrotal reactions were produced in early passages in guinea pigs, but in later passages some animals developed swelling and erythema of the scrotum The strain was lethal for chick embryos 4 to 6 days following inoculation

Parker et al (1951) reported that the sera from 8 of 10 snowshoe rabbits that were trapped and brought to the laboratory were positive for spotted fever by the complement-fixation test, and *Haemaphysalis leporis-palustris* (H-l-p) ticks picked from 3 of the rabbits that showed a positive serologic reaction were shown to contain rickettsiae of the spotted-fever type Likewise, Philip and Hughes (1953) reported that the sera of 8 of 127 black-tailed jack rabbits (*Lepus californicus deserticola*) collected in Nevada in 1951, and 27 of 133 similar sera collected in 1952, were positive for spotted-fever type rickettsiae by the complement-fixation test. In addition, certain of the sera obtained in 1952 that showed positive complement-fixation reactions against spotted-fever antigen were subjected to toxin-neutralization tests in mice (cf. Bell and Pickens, 1953) Four of 8 sera were also positive by this test in serum dilutions between 1 8 and 1 64 Of 5 other sera, which were negative in complement-fixation tests, 2 showed positive neutralization titers of 1 16 and 1 32, respectively (Philip et al, 1955) These data, of course, indicate that rabbits other than cottontails are of importance in the spotted fever problem In Brazil, the opossum (*Didelphus paraguayensis* and *D aurita* in São Paulo, and *D marsupialus* in Minas Gerais) has been found naturally infected (Travassos, 1948) Magalhães and Rocha (1942) not only found dogs susceptible to experimental infection with Brazilian spotted fever, but also found them naturally infected Travassos (1948) inoculated guinea pigs with blood from an apparently normal dog that lived in a spotted fever endemic area and found the guinea pigs to be subsequently immune to spotted fever challenge Experimentally, the dog and the opossum (*D aurita*) develop inapparent infections with the São Paulo strain, and viable rickettsiae persist in these hosts for long periods of time Six serial passages of the São Paulo strain through opossums (*D aurita*) resulted in a loss of virulence of the agent The Brazilian cavy (*Cavia aperea*), the wild rabbit (*Sylvilagus minensis*), the domestic dog and the opossum (cited above) have all been shown to be naturally infected, the Brazilian plains dog, capybara (*Hydrochoerus capybara*), coati and certain kinds of bats are susceptible (Dias and Martins, 1939) In Colombia, Patiño-Camargo (1941) showed the native monkey, *Cebus fatuelles*, to be susceptible

Most large domestic animals are insusceptible However, Badger (1933) found dogs and sheep mildly susceptible to experimental infection Older dogs showed no clinical manifestations, although rickettsiae were recovered from their blood on the 4th, the 6th and the 8th days following inoculation A grown dog, reared in an endemic spotted-fever area, was apparently immune. A puppy reacted to experimental inoculation with fe-

troencephalographic abnormalities Abnormal electroencephalograms were seen in a high proportion of patients who had fever for more than 10 days. Berlin and Thomas (1948) likewise have reported that occasional patients may show permanent severe neurologic sequelae eventually resulting in death Even in mild cases, convalescence is slow, and complete recovery from severe infection may require several months to a year or longer.

Harrell (1949), in his excellent review, mentioned that myocardial failure may result from the pathologic process or from overloading the circulation by intravenous therapy Along these lines, Aquilina et al. (1952) have reported what may be the first case of Rocky Mountain spotted fever complicated by an intermittent nodal tachycardia. This condition is apparently associated with extensive myocardial damage It is generally considered that persons having recovered from spotted fever are permanently immune.

Pathologic Picture

[illegible]

2nd week after infection The spotted fever rickettsiae can be demonstrated fairly readily in sections, and their localization determines the sites of lesions (Wolbach, 1948). The rickettsiae first invade the nuclei of capillary endothelial cells, where they multiply in great numbers and destroy the cells The lesion then extends centripetally along the intima into slightly larger vessels (arterioles), where smooth muscle cells of the media are also invaded and destroyed (Lillie, 1941) The destruction of smooth muscle cells is a most distinctive feature of Rocky Mountain spotted fever With the death of the cells, necrosis occurs in the intima and the media of the blood vessels, causing thrombosis and extravasation of blood. Microinfarcts are then formed, chiefly in the skin, the subcutaneous tissues and the central nervous system. As the disease develops, perivascular accumulations of mononuclear macrophages appear, and the vascular lesions assume a proliferative or granulomatous character. According to Wolbach (1948), "the necrosis of skin in Rocky Mountain spotted fever occurs independently of stasis produced by pressure. Its common sites are scrotum, prepuce, fingers, toes and ear lobes; rarely, the soft palate." In the central nervous system, areas of demyelination may be found adjacent to or removed from the vascular lesions. Rocky Mountain spotted fever without question produces greater damage to the skin, the subcutaneous tissues and the brain than does any other rickettsial disease. Lillie has reported (1941) that the pathologic picture of spotted fever in the Rocky Mountain area and in the eastern United States is essentially the same

One feature of spotted fever, which cannot be emphasized too strongly, is that it may be duplicated exactly in experimental animals, Wolbach, 1919. Guinea pigs sacrificed for examination at the height of the infection show edema and hemorrhages in the skin and the subcutaneous tissues of the scrotum, the paws and the ears. Inguinal and axillary lymph nodes are swollen and reddened The spleen ranges from 3 to 5 times larger than normal and is dark red and firm in consistency; occasionally, there is a very thin, translucent layer of fibrin upon its surface The most striking changes are found in the testes and the adnexa. The former are swollen and markedly injected, usually with minute hemorrhages in the tunica at both poles Small hemorrhages are also found in the polar fat, the epididymus, in the subcutaneous tissues surrounding the anus and the scrotum and often in the cremasteric muscles and the parietal tunicae, which are deep red and adherent, not only to each other but to the testes Later in the course of the disease, the testes become adherent to the scrotum, and the skin of the scrotum, the paws and the ears frequently shows necrosis The central nervous system may be injected but shows no gross lesions. The histologic character of individual lesions in the brain does not differ appreciably from those caused by typhus except that, in spotted fever, a higher proportion of the focal lesions are found in the pons, the medulla, the midbrain and the cerebellum.

Experimental Infection; Host Range

Man is entirely an incidental victim to spotted fever and is in no way responsible for the maintenance of the infection in nature, which is largely due to ticks and the animals on which they feed. The epidemiologic evidence in North America clearly points to certain small animals belonging to the many groups of rodents which serve as hosts to the immature stages of the tick vectors Dogs also are involved in the eastern United States,

ring in pairs and often surrounded by a very narrow but definite clear zone, or halo, as if encapsulated. The distal ends of the pairs are often tapered, so that they resemble minute pneumococci The rickettsiae average about 1 μ in length and from 0 2 to 0 3 μ in width They stain best by special methods with Giemsa stain, the rickettsiae take a purplish tinge; with the Castaneda method they have a light blue appearance, whereas with the Macchiavello method,* a most satisfactory stain for rickettsiae, they are stained a bright pink or red against a bluish background

Figure 128 shows spotted fever rickettsiae in a stained smear of infected yolk sac material. Like other rickettsiae, *R. rickettsii* is gram negative All attempts to cultivate *R rickettsii* on artificial or cell-free media have been unsuccessful, but they grow readily in tissue cultures and in the chorio-allantois and the yolk sac of the developing chick embryo (Cox, 1938, 1941, 1948) The rickettsiae of the spotted fever group (*R rickettsii* and *R conorii*), in contrast with the typhus rickettsiae (*R. typhi* and *R. prowazekii*), continue to grow in the chorio-allantoic membrane and the yolk sac of eggs placed at room temperature for 2 or 3 days after the death of the embryo A similar observation has been reported by Bell and Pickens (1953) in their studies on a toxic substance associated with spotted fever rickettsiae The striking feature of *R rickettsii* in tick tissues and plasma tissue cultures is its apparent preference for the cell nuclei where it grows in compact clusters (Pinkerton and Hass, 1932) Often the entire nucleus becomes distended with organisms There is a definite peripheral conden-

* Macchiavello's stain Fix slide in flame Flood smear with 0 5 per cent solution of aqueous basic fuchsin of pH 7.2 to 7 5 Stain for 5 minutes Rinse rapidly with 0 5 per cent citric acid solution Wash thoroughly with tap water Counterstain with 1.0 per cent aqueous methylene blue for 1 or 2 minutes Rinse well with tap water and examine when dry

Fig 128 Stained preparation of yolk-sac material from infected chick embryo (×1245)

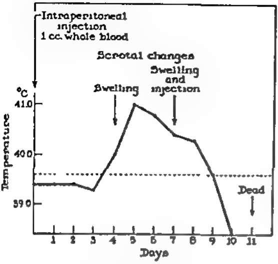

FIG. 127 Temperature curve of a guinea pig infected with the Bitter Root strain of Rocky Mountain spotted fever.

ver and respiratory symptoms, and rickettsiae were recovered from its blood on the 4th day. Infected *D andersoni* were fed on 2 puppies which, after incubation periods of 5 and 6 days respectively, developed signs of illness, and rickettsiae were recovered from the blood of each Rickettsiae were recovered from the blood of a young sheep on the 4th, the 6th, the 8th and the 10th days after inoculation Miller (1950) was unable to induce apparent infection in 6 foxes and 2 raccoons by inoculating them with a highly virulent strain of rickettsiae; however, the animals developed complement-fixing antibodies. Dogs, foxes and raccoons, on which *D. variabilis* nymphs and adults were found under natural conditions on Long Island, N. Y., showed high titers for spotted fever complement-fixing antibodies.

The guinea pig is the most suitable common laboratory animal for experimental purposes The guinea pig's temperature usually does not rise until 3 or more days have passed after the inoculation of blood from human patients, and generally a few guinea pig passages are required before the incubation period becomes fixed at 2 or 3 days (Fig 127). The febrile period may last from 5 to 14 days Death, which usually occurs with well-established strains on the 6th to the 8th day of fever, is preceded by a sudden drop in temperature to subnormal. If a guinea pig recovers, its temperature begins to drop at the end of 7 or 8 days and gradually reaches normal within 3 to [illegible] days The first sign of the disease in male guinea pigs is swelling and reddening of the scrotal skin on the 3rd or the 4th day of fever (Fig 127). At this time, the animal shows signs of discomfort, loss of appetite and roughening of the coat. The scrotal reaction may develop into a necrotic condition, followed by sloughing and subsequent healing with scar formation. Necrosis and sloughing of the ears and the foot pads also occur frequently. Rabbits rarely die of the disease, although they develop fever and may show ear and scrotal reactions similar to those seen in guinea pigs. Rabbits develop antibodies detectable by the Weil-Felix reaction. The course of the disease in monkeys may be very rapid, with early death occurring with highly virulent strains. Monkeys frequently develop a rash on the face, over the lower back and on the thighs. Swelling and redness of the scrotum is common Necrosis of the ears also occurs Like rabbits, monkeys develop antibodies detectable by the Weil-Felix reaction. White rats are moderately susceptible to infection. In tests carried out in a series of white rats, white mice and ground squirrels to determine the persistence of spotted-fever infection, the only definitely positive test was obtained with the brain of a white rat sacrificed 30 days after intraperitoneal inoculation (Philip and Parker, 1938). No definite positive result was obtained in any of similar tests carried out to determine the persistence of tick-borne typhus. Concerning persistence of infection in man, it is worthy of note that Parker et al. (1954) recovered viable rickettsiae from the lymphatic tissues of a colored male 1 year following his recovery from Rocky Mountain spotted fever. Even more remarkable is the fact that the recovery was made from the lymph node tissue after it had been stored for 29 months in a dry-ice chest.

Etiology

The distribution and the morphology of *R. rickettsii* are identical in the tissues of man, monkey, rabbit and guinea pig, Wolbach, 1919. In tissue sections, the rickettsiae appear as minute organisms, frequently occur-

to interfere with the virulent rickettsiae The most protective material produced by various fractionation procedures contained protein, lipid, carbohydrate, less than 0 1 per cent phosphorus, no pentosenucleic acid and no deoxyribosenucleic acid The rickettsial interfering substance is highly specific, the interference phenomenon is apparently independent of the time that the challenge dose is given, provided that it follows the protective dose by no longer than approximately 10 days

Diagnosis

In spite of the commonly expressed opinion that spotted fever is an easily recognized infection, even those familiar with the disease may make errors in diagnosis The very mild infections and the rapidly fulminating types are quite difficult to diagnose clinically Furthermore, in areas where both spotted fever and murine (endemic) typhus are present, an additional difficulty is encountered because of their marked clinical similarity Rocky Mountain spotted fever should be suspected in cases of febrile disease that occur during the tick season in those persons whose occupations or habits expose them to ticks Close examination may show the presence of a tick or an indurated area representing the site of a tick-bite The differential diagnosis is difficult, since it entails consideration of certain exanthematous diseases, as well as other rickettsial diseases and tick-borne infections Rocky Mountain spotted fever rash may resemble that of measles, meningococcal meningitis, scarlet fever, smallpox, typhoid fever and septicemic conditions as well as certain drug rashes Measles is the disease most frequently confused with spotted fever

In the United States, the other rickettsial diseases likely to cause difficulty in diagnosis are rickettsialpox and murine typhus Rickettsialpox (see below), transmitted to man by means of a mite, has been found thus far only in urban areas of the east and is characterized by an initial lesion (eschar) at the site of the mite-bite Murine typhus is transmitted to man by a flea-bite It occurs mainly in the late summer and fall in the southern and south-eastern states and is characterized by the development of a rash, first on the trunk and later on the extremities The rash in murine typhus is usually much less noticeable and of shorter duration than that of spotted fever The two tick-borne diseases which must be considered are Colorado tick fever and tularemia There is usually no rash during Colorado tick fever The development of an ulcer at the site of tick attachment, accompanied by enlargement of the regional lymph nodes, suggests tularemia

The infection test, the Weil-Felix reaction, the protection or virus neutralization test and the complement-fixation test are ordinarily used for diagnosis In addition, a toxic substance (Bell and Pickens, 1953) and serologically active *erythrocyte-sensitizing* substances (ESS) have been demonstrated (Chang et al, 1954) for several members of the spotted-fever group of organisms Both of these findings are important because they have not only increased our knowledge about certain basic properties of the organisms, but because they could well be put to practical use for diagnostic purposes

In the infection test, male guinea pigs are inoculated intraperitoneally with blood from a suspected patient Clotted blood, plasma, serum or, preferably, whole citrated blood may be used Once the disease is established, it may be maintained by inoculating normal male guinea pigs intraperitoneally with blood, splenic tissue or testicular washings taken from an infected guinea pig on the 2nd or the 3rd day of fever By establishing the disease in guinea pigs, it is possible to apply cross-immunity tests with known strains of spotted fever rickettsiae or other infectious agents

The Weil-Felix reaction, which consists of testing a patient's serum for agglutinins against Proteus organisms, aids in limiting the probable diagnosis to the rickettsial group of diseases, but it is of no certain aid in differentiating spotted fever from typhus In using the Weil-Felix test, it is desirable that at least 2 blood samples be used one taken as soon as spotted fever is suspected, the other approximately 2 weeks after the onset The first blood sample is seldom diagnostic and is valuable chiefly as a reference point in determining whether there is a subsequent rise in titer in the second specimen A titer of less than 1 320 cannot be considered definitely diagnostic In the majority of spotted-fever cases the titers for OX-19 agglutinins are highest, but occasionally, particularly with sera from patients in certain areas of Wyoming (Parker, 1938), those for

sation of the nuclear chromatin, similar to that seen in association with the intranuclear inclusions of certain virus diseases. The unique intranuclear localization of *R. rickettsii* was used by Pinkerton (1936) in the classification of atypical strains which gave ambiguous cross-immunity reactions Spotted fever rickettsiae do not pass Berkefeld V, N or W candles or Seitz filter pads They are killed in a few minutes by exposure to moist heat at 50° C or to chemical agents, and in a few hours by desiccation at room temperature Red and white blood cells from infected guinea-pig blood retain their infectivity even after repeated washings At room temperature guinea pig blood retains its infectivity for only about a week, but in a cold room it remains infectious for about 2 weeks Infected guinea pig brain and spleen, suspended in glycerol stored in sealed containers at −70° C or in a dry-ice box, remain infectious for periods up to a year

In 1953, Price reported his observations on an interference phenomenon which was observed for the first time with rickettsial infections He used two strains of Rocky Mountain spotted fever one of low virulence (T strain) and one of high virulence (R strain). Both strains were shown to possess toxin (cf. Bell and Pickens, 1953), a hemolytic factor, and to be practically identical immunologically. It was found that the avirulent T strain protected guinea pigs when both strains were given simultaneously, or when the R strain was given as much as 14 days later, it interfered with the R strain when the T strain had been inactivated by ultraviolet irradiation but not by heat; and it protected animals against tick-borne typhus. The protective property was shown to be a part of the rickettsiae and not some other substance present in the infected yolk sac suspensions. Suspensions of rickettsiae causing Q fever, scrub typhus, epidemic and murine typhus which are immunologically unrelated to Rocky Mountain spotted fever likewise were found to protect guinea pigs against an injection of the virulent R strain of spotted fever. However, the inoculated animals did develop the typical symptoms caused by the different rickettsiae used to protect them against spotted fever, but the spotted fever rickettsiae found in the testes-tunica tissues were stated to be at least 1,000 times fewer than those found in control animals. In another interesting experiment, ground squirrels, field mice (*Microtus*) and cottontail rabbits were infected by exposure to many *D. andersoni* containing a mildly virulent strain of spotted fever and a few *D. andersoni* containing a highly virulent spotted fever strain. Uninfected ticks allowed to feed upon these animals were found to contain only the mildly virulent strain This experimental finding may help to explain some of the interesting epidemiologic observations that, in certain localized areas, Rocky Mountain spotted fever strains having either high or low virulence persist in ticks year after year.

In a subsequent paper, Price et al (1954) presented data indicating that the rickettsial-interference phenomenon is not due to competition between the rickettsiae for the same susceptible host cells. Guinea pigs injected intraperitoneally with highly virulent rickettsiae of the R strain of spotted fever, concentrated and inactivated with ultraviolet light, were found to be protected when challenged 3 hours later with highly virulent rickettsiae of the same strain The protection seemed to be dependent on the ratio of interfering dose to infecting dose and not on the number of susceptible host cells Thus, injections of 10^6 ultraviolet inactivated rickettsiae gave no protection to guinea pigs even when the challenge dose was only 100 living virulent rickettsiae However, 10^7 ultraviolet inactivated rickettsiae did confer some protection against as many as 10,000 challenge doses If the living, virulent rickettsiae were given 3 hours before the inactivated rickettsiae, little, if any, protection was observed in any case A chemical component was isolated from suspensions of the virulent R strain of rickettsiae by a procedure that involved purification by the celite and albumin process, and treatment in a sonic vibrator for 30 minutes. It caused no detectable symptoms of illness and was found to protect from 80 to 90 per cent of guinea pigs against a challenge of virulent rickettsiae given 3 hours later. Rickettsial suspensions that were prepared in a similar manner but without sonic vibration treatment showed no interference or protective effect. Concentrated preparations of the soluble antigen of *R. rickettsii* had no ability

to interfere with the virulent rickettsiae. The most protective material produced by various fractionation procedures contained protein, lipid, carbohydrate, less than 0.1 per cent phosphorus, no pentosenucleic acid and no deoxyribosenucleic acid. The rickettsial interfering substance is highly specific; the interference phenomenon is apparently independent of the time that the challenge dose is given, provided that it follows the protective dose by no longer than approximately 10 days.

Diagnosis

In spite of the commonly expressed opinion that spotted fever is an easily recognized infection, even those familiar with the disease may make errors in diagnosis. The very mild infections and the rapidly fulminating types are quite difficult to diagnose clinically. Furthermore, in areas where both spotted fever and murine (endemic) typhus are present, an additional difficulty is encountered because of their marked clinical similarity. Rocky Mountain spotted fever should be suspected in cases of febrile disease that occur during the tick season in those persons whose occupations or habits expose them to ticks. Close examination may show the presence of a tick or an indurated area representing the site of a tick-bite. The differential diagnosis is difficult, since it entails consideration of certain exanthematous diseases, as well as other rickettsial diseases and tick-borne infections. Rocky Mountain spotted fever rash may resemble that of measles, meningococcal meningitis, scarlet fever, smallpox, typhoid fever and septicemic conditions as well as certain drug rashes. Measles is the disease most frequently confused with spotted fever.

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The infection test, the Weil-Felix reaction, the protection or virus neutralization test and the complement-fixation test are ordinarily used for diagnosis. In addition, a toxic substance (Bell and Pickens, 1953) and serologically active erythrocyte-sensitizing substances (ESS) have been demonstrated (Chang et al., 1954) for several members of the spotted-fever group of organisms. Both of these findings are important because they have not only increased our knowledge about certain basic properties of the organisms, but because they could well be put to practical use for diagnostic purposes.

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OX-2 agglutinins are highest. The Proteus agglutinins usually appear toward the end of the 2nd week of the disease, but sometimes they do not appear until early convalescence; in some patients, no Proteus agglutinins are produced.

According to Parker (1938), the protection or virus-neutralization test is nearly always of diagnostic value. As performed in his laboratory, duplicate mixtures are prepared, each containing 0.5 cc. of serum and 0.1 cc., 0.25 cc. and [illegible]5 cc. of serum virus, respectively. The mixtures are held at room temperature for 30 minutes and then injected intraperitoneally into guinea pigs. Control animals receive the same amount of infectious serum mixed with normal serum. The most consistent results are obtained with blood samples taken in convalescence, although some sera taken during lysis of the disease show definite neutralizing capacity. The neutralization test is claimed to be of greater value than the agglutination reaction in testing blood specimens from relatively mild cases and may give even better results than the infection test

The complement-fixation test is an additional laboratory aid and has the distinct advantage over the Weil-Felix reaction that it may be made highly specific and used to differentiate Rocky Mountain spotted fever from epidemic typhus, murine typhus, Q fever and scrub typhus as well as other members of the spotted-fever group. Satisfactory antigens may be prepared from rickettsiae cultivated by the agar-tissue culture method of Zinsser, et al. (1939) or by the yolk-sac method of Cox (1938) Spotted-fever and tick-borne typhus rickettsiae contain soluble [illegible]

removed, by subjecting the rickettsiae to repeated washings, and the resulting washed rickettsiae provide highly specific antigens (Plotz et al., 1944). Plotz (1945), using purified antigens prepared from rickettsiae of Rocky Mountain spotted fever, boutonneuse fever, South African tick-bite fever and Bra- [illegible]

identical and that boutonneuse and South African tick-bite fever (both now included in tick-borne typhus) are antigenically related. There was no reciprocal cross-fixation, however, between Rocky Mountain or Brazilian spotted fever and boutonneuse or South African tick-bite fever. Pereira and Travassos (1951) likewise have reported the rickettsiae of Rocky Mountain spotted fever and Brazilian spotted fever (São Paulo typhus) to be antigenically identical by using complement-fixation tests with washed rickettsial suspensions. Silva-Goytia and Elizondo (1952), using [illegible] tity b[illegible] strai[illegible] spotted fever strains (Michoacan and La Laguna strains I and II). The latter workers support the suggestion of Bustamente and Varela (1943) that the several colloquial names for spotted fever infections in various parts of the western hemisphere be combined under the term, American spotted fever. Since the word "American" is considered in many quarters of the world to pertain to the United States of America, the author suggests that perhaps a more appropriate and realistic term would be "Western Hemisphere" or "New World" spotted fever.

Van der Scheer et al. (1947) prepared purified soluble antigens from spotted-fever [illegible]

procedures commonly used in performing complement-fixation tests. They found the modified Kolmer test used by Van der Scheer et al (1947) to possess the advantages of being specific, sensitive and already essentially familiar to workers in most public health laboratories.

The Weil-Felix reaction and the protection test are of no value in testing for long-past infections. The agglutinin titer for Proteus strains falls rapidly after recovery is complete, and it is unusual for a patient's serum to show neutralizing capacity [illegible] year after illness However, complement-fixing antibodies usually appear during the 2nd week of illness and persist for at least 6 to 8 years Some comment should be made at this point, however, about the effect of antibiotic therapy upon the development of specific, rickettsial complement-fixing antibodies. Wong and Cox (1948) found that chlortetracycline, administered to guinea pigs at very early stages of experimental rickettsial infections, completely suppressed the production of complement-fixing antibodies. Harrell (1949) likewise indicated that the administration of chlortetracycline or chloramphenicol to man during the

early stages of illness interfered with the development of complement-fixing antibodies to Rocky Mountain spotted fever Schubert (1952) reviewed the literature dealing with this subject and pointed out that therapy with chlortetracycline or chloramphenicol apparently had less effect upon the development of Proteus agglutinins than upon the production of complement-fixing antibodies Lackman and Gerloff (1953) confirmed the fact that caution must be exerted in interpreting negative findings in complement-fixation tests in cases of Rocky Mountain spotted or typhus fever when the patient has been treated with chlortetracycline or chloramphenicol The Weil-Felix test appears to be of most value in such cases

In 1953, Bell and Pickens reported the occurrence in infected yolk sacs of specific toxic substances lethal for mice, associated with 10 strains of spotted-fever group rickettsiae including the agents of Rocky Mountain spotted fever (2 western Montana strains, a Minnesota and an Iowa strain), Brazilian spotted fever, boutonneuse fever South African tick-bite fever, Indian tick typhus and 2 strains of spotted-fever rickettsiae isolated from ticks, *A maculatum* and *H leporis-palustris*, respectively Attempts to demonstrate a toxin for *R. akari* (rickettsialpox) and *R australis* (Queensland tick typhus) were unsuccessful Toxin was neutralized in relatively high titer by sera from human beings and guinea pigs convalescent from spotted fever but not neutralized by sera from normal humans or guinea pigs or by sera from guinea pigs previously infected with epidemic typhus, murine typhus or Q fever The toxin produced by *R rickettsii* was found to be neutralized also by the sera of human beings and guinea pigs that had been vaccinated with spotted-fever tick vaccine This is a most interesting finding, since no satisfactory test for measuring the response of individuals to vaccination had been recognized previously, complement-fixation tests on such individuals thus far have always given inconclusive results Preliminary tests have also indicated that the toxin neutralization test can differentiate between the rickettsiae of Rocky Mountain spotted fever and of tick-borne typhus (boutonneuse fever) This finding suggests the possibility that the toxin neutralization test may be of value also in distinguishing between even more closely related strains of rickettsiae within the spotted fever group which differ from *R rickettsii* with respect to virulence or host species, e g., those strains isolated from *H leporis-palustris*, *D parumapertus* and *A maculatum*. While it has been shown that the complement-fixation test serves to differentiate between certain strains of spotted-fever rickettsiae, it has failed to do so between others (Parker et al , 1939) Certainly, the interesting possibilities promised by the toxin-neutralization test should be pursued to the fullest extent in order to elucidate further our knowledge concerning this most interesting group of organisms

In 1954, Chang et al reported that serologically active erythrocyte-sensitizing substances (ESS) were isolated from yolk-sac membranes infected with several rickettsiae of the Rocky Mountain spotted-fever group Long Island and R strains of Rocky Mountain spotted fever, the MK strain of rickettsialpox a North African strain of tick-borne typhus (boutonneuse fever) and a strain of South African tick-bite fever (also tick-borne typhus) Preliminary tests made with Queensland tick typhus failed to show ESS, but the tests were not conclusive Tests with immune sera indicated that the ESS obtained from all the above rickettsial strains were probably common and group specific Thus, sera from rickettsialpox and Rocky Mountain spotted fever patients agglutinated erythrocytes treated with rickettsialpox-ESS, Rocky Mountain spotted fever-ESS and boutonneuse fever-ESS to approximately the same titer. Human postvaccination sera gave weakly positive or negative tests Most rabbit convalescent sera agglutinated erythrocytes treated with rickettsialpox-ESS, Rocky Mountain spotted fever-ESS and boutonneuse fever-ESS Convalescent guinea pig sera gave irregular results. The ESS test, which appears to be somewhat more sensitive and simpler than the complement-fixation test, compared favorably with the complement-fixation and the Weil-Felix tests in the detection of early antibodies in studying 20 cases of Rocky Mountain spotted fever and 5 cases of rickettsialpox. ESS preparations made from yolk sacs infected with rickettsialpox were found to be stable at 4° C. for at least 1 year. Oc-

OX-2 agglutinins are highest. The Proteus agglutinins usually appear toward the end of the 2nd week of the disease, but sometimes they do not appear until early convalescence; in some patients, no Proteus agglutinins are produced.

According to Parker (1938), the protection or virus-neutralization test is nearly always of diagnostic value. As performed in his laboratory, duplicate mixtures are prepared, each containing 0.5 cc. of serum and 0.1 cc., 0.25 cc. and 0.5 cc. of serum virus, respectively. The mixtures are held at room temperature for 30 minutes and then injected intraperitoneally into guinea pigs. Control animals receive the same amount of infectious serum mixed with normal serum. The most consistent results are obtained with blood samples taken in convalescence, although some sera taken during lysis of the disease show definite neutralizing capacity. The neutralization test is claimed to be of greater value than the agglutination reaction in testing blood specimens from relatively mild cases and may give even better results than the infection test.

The complement-fixation test is an additional laboratory aid and has the distinct advantage over the Weil-Felix reaction that it may be made highly specific and used to differentiate Rocky Mountain spotted fever from epidemic typhus, murine typhus, Q fever and scrub typhus as well as other members of the spotted-fever group. Satisfactory antigens may be prepared from rickettsiae cultivated by the agar-tissue culture method of Zinsser, et al. (1939) or by the yolk-sac method of Cox (1938). Spotted-fever and tick-borne typhus rickettsiae contain soluble antigens which give cross-fixation respectively with tick-borne typhus or spotted-fever antisera. However, the soluble antigens may be removed, by subjecting the rickettsiae to repeated washings, and the resulting washed rickettsiae provide highly specific antigens (Plotz et al., 1944). Plotz (1945), using purified antigens prepared from rickettsiae of Rocky Mountain spotted fever, boutonneuse fever, South African tick-bite fever and Bra-

There was no reciprocal cross-fixation, however, between Rocky Mountain or Brazilian spotted fever and boutonneuse or South African tick-bite fever. Pereira and Travassos (1951) likewise have reported the rickettsiae of Rocky Mountain spotted fever and Brazilian spotted fever (São Paulo typhus) to be antigenically identical by using complement-fixation tests with washed rickettsial suspensions. Silva-Goytia and Elizondo (1952), using similar methods, showed complete identity between a Rocky Mountain spotted fever strain (Bitter Root Valley) and 3 Mexican spotted fever strains (Michoacan and La Laguna strains I and II). The latter workers support the suggestion of Bustamente and Varela (1943) that the several colloquial names for spotted fever infections in various parts of the western hemisphere be combined under the term, American spotted fever. Since the word "American" is considered in many quarters of the world to pertain to the United States of America, the author suggests that perhaps a more appropriate and realistic term would be "Western Hemisphere" or "New World" spotted fever.

Van der Scheer et al. (1947) prepared purified soluble antigens from spotted-fever rickettsiae which were highly specific and gave little or no fixation with syphilitic human sera. Schubert et al. (1951) compared 5 procedures commonly used in performing complement-fixation tests. They found the modified Kolmer test used by Van der Scheer et al. (1947) to possess the advantages of being specific, sensitive and already essentially familiar to workers in most public health laboratories.

The Weil-Felix reaction and the protection test are of no value in testing for long-past infections. The agglutinin titer for Proteus strains falls rapidly after recovery is complete, and it is unusual for a patient's serum to show neutralizing capacity a year after illness. However, complement-fixing antibodies usually appear during the 2nd week of illness and persist for at least 6 to 8 years. Some comment should be made at this point, however, about the effect of antibiotic therapy upon the development of specific, rickettsial complement-fixing antibodies. Wong and Cox (1948) found that chlortetracycline, administered to guinea pigs at very early stages of experimental rickettsial infections, completely suppressed the production of complement-fixing antibodies. Harrell (1949) likewise indicated that the administration of chlortetracycline or chloramphenicol to man during the

ally become afebrile within 48 to 72 hours after initiation of therapy, so that it can be discontinued after 4 to 6 days However, if a relapse occurs, another course of antibiotics may be given, since there is no evidence that the rickettsiae develop drug resistance Recently, reports have appeared indicating that a combination of specific antibiotic therapy with supportive hormone therapy (cortisone), gives promise of modifying further the severe effects of Rocky Mountain spotted fever as well as shortening its course (Aikawa and Harrell, 1953).

Arney (1952) treated a comatose patient with 500 mg. of chlortetracycline in 500 ml of 5 per cent glucose intravenously and 100 mg of cortisone intramuscularly every 6 hours for 3 doses Twelve hours later the intravenous administration of chlortetracycline was repeated This dosage was repeated twice within the next 24 hours, then once daily for 3 days The patient's clinical picture improved within 6 hours, and the improvement was continuous Within 18 hours after starting chlortetracycline and cortisone therapy, she was able to take fluids by mouth The temperature returned to normal after 24 hours Her convalescence from then on was uneventful Similarly, Workman et al (1952) reported that 9 cases infected with Rocky

TABLE 37 INCIDENCE OF ROCKY MOUNTAIN SPOTTED FEVER IN THE WESTERN HEMISPHERE*

COUNTRY	PERIOD	NO OF CASES
United States (by area)		
Pacific Coast States		
Washington Oregon and California	1947 1954	101
Mountain States		
Idaho, Montana, Wyoming Nevada Utah, Colorado, Arizona and New Mexico	" "	531
West North-Central States		
North and South Dakota, Minnesota Nebraska, Iowa, Kansas and Missouri	" "	97
West South-Central States		
Oklahoma, Arkansas, Texas and Louisiana	" "	296
East North-Central States		
Wisconsin, Michigan, Illinois, Indiana and Ohio,	" "	347
East South-Central States		
Kentucky, Tennessee, Mississippi and Alabama	" "	499
New England States		
Maine, Vermont, New Hampshire, Massachusetts, Rhode Island and Connecticut	" "	11
Middle Atlantic States		
New York, New Jersey and Pennsylvania	" "	396
South Atlantic States		
Delaware, Maryland, West Virginia, Virginia, North and South Carolina, Georgia and Florida	" "	2 239
United States—(total)	1947-1954	4,614
Canada	1947-1956	3
Mexico	1947-1956	75
Panama	1950-1956	4
Colombia	1950 1956	362
Brazil	1948-1954	41

* Statistics for United States from Cawley & Wheeler (1957) Statistics for countries other than United States courtesy of Ruth R Puffer (1957)

casionally, a slight antigenic overlap was found in the ESS tests between Rocky Mountain spotted fever and typhus, but otherwise clear-cut differentiation was observed. These observations certainly merit further study.

Treatment

In the early days, the only treatment for spotted fever was good nursing care and symptomatic treatment Metaphen, sulfanilimide, sulfapyridine, penicillin and streptomycin have been tried with no definite evidence of benefit The sulfonamides are not only useless but actually harmful Numerous reports in the early literature described attempts to produce an immune serum for treatment The most promising work along these lines was that of Topping (1943) who used hyperimmune sera prepared in rabbits by injecting them with living rickettsiae obtained from infected yolk sacs or ticks. The results obtained in a small number of patients indicated that serum treatment reduced the case fatality rate if it was administered before the 3rd day of rash Para-aminobenzoic acid (PABA) has been found useful in the treatment of spotted fever, based on the original work of Snyder et al (1942), who demonstrated in white mice that oral administration of PABA reduced the mortality from experimental murine typhus It has been reported to give favorable results in spotted-fever-infected guinea pigs (Anigstein and Bader, 1945), in chick embryos (Hamilton, 1945), and in human beings (Rose, et al, 1945, Ravenel, 1947) It is now a matter of record, however, that the problem of treatment of spotted fever as well as of all other rickettsial diseases essentially has been solved, for there are now available at least 3 antibiotics—chlortetracycline, chloramphenicol and oxytetracycline—which have been proved to be of great value not only in the laboratory but also by repeated clinical test Each of these antibiotics has been used with remarkable results and each has been found to be far superior to immune serum or para-aminobenzoic acid In addition, tetracycline has been shown to be quite effective in the treatment of epidemic typhus fever, and there is every reason to believe that it would be equally beneficial in the treatment of diseases of the spotted-fever group (Yanez, 1957)

Extensive laboratory studies with chlortetracycline have been reported by Wong and Cox (1948) and Anigstein et al. (1948), on the use of chloramphenicol by Smadel and Jackson (1947) and Smadel et al. (1949); and on the efficacy of oxytetracycline by Snyder et al. (1950), Smadel et al (1950) and Rose (1950). Numerous clinical tests carried out by competent observers have fully substantiated the laboratory results

For therapy with chlortetracycline see Cooke (1948), Havens and Stickney (1950), Ross et al (1948); for results with chloramphenicol, see Pincoffs et al (1948), Parker et al. (1950); for oxytetracycline, see Powell et al. (1951). Good reviews on the subject have been written by Finland et al. (1949), Rose and Kneeland (1949), Smadel (1949, 1951) and Harrell (1952).

All of the above-named antibiotics are rickettsiostatic in their action and not rickettsiocidal. The antibiotics suppress the growth of the rickettsiae while the patient overcomes the disease by developing immunity and consequent control of the infectious agent After recovery, the host may continue to harbor living rickettsiae for considerable periods of time The matter of drug dosage for any of the rickettsioses, including spotted fever, is relatively simple and essentially the same The administration of 2.0 to 3 0 Gm orally, in divided doses, each day until the patient's temperature has been normal for 48 to 72 hours is a good general rule to follow Smadel (1951) has recommended an initial loading dose of 3 0 Gm when chloramphenicol is used It is not necessary to use such a loading dose for chlortetracycline or oxytetracycline In the case of chlortetracycline it is recommended that the daily amount of drug given orally be approximately 100 mg. per 10 pounds body weight; the total amount should be divided and given at 3- to 4-hour intervals The antibiotics are most effective when they are given early in the course of the illness Thus, it is of utmost importance to be aware of the possibility of spotted fever in any area where ticks occur even though no human cases have been reported It is important to keep in mind, too, that antibiotics prevent further damage caused by the action of the organism but do not repair damage that has already occurred Spotted-fever patients usu-

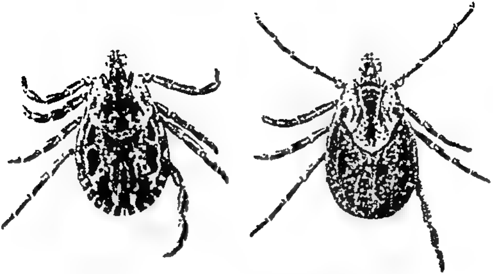

FIG. 129 *Dermacentor variabilis* (*Left*) Male (*Right*) Female (Photograph from Dr R. A Cooley, U S Public Health Service, Hamilton, Mont)

FIG 130 *Dermacentor andersoni* (*Left*) Male (*Right*) Female (Photograph from Dr R A. Cooley, U S Public Health Service, Hamilton, Mont)

adults averages about 80 per cent and for children about 37 5 per cent A high case fatality rate also prevails in other parts of western M[illegible]

spotted fever in the East is about as fatal as it is in the West In comparing data for a 10-year period (1930-1939 inclusive), Topping (1941) reported that the crude fatality rate for Idaho and Montana was 28 1 per cent, while for Maryland and Virginia it was [illegible] 4 per cent Little difference was found in the fatality rates when the two areas were compared on the basis of age In the western states, one half of the cases (50 2%) occurred in persons aged 40 years or over, while in the eastern states this was practically reversed with the largest number (46 8%) occurring in persons under 15 years of age. As

TABLE 38 ROCKY MOUNTAIN SPOTTED FEVER TALLY IN THE UNITED STATES, 1947-56*

YEAR	NO. OF CASES
1947	596
1948	547
1949	570
1950	464
1951	347
1952	327
1953	313
1954	294
1955	295
1956	277

* Statistics from Dauer (1957)

Mountain spotted fever gave a better response with a combined treatment of chloramphenicol and cortisone than would be expected with chloramphenicol alone Cortisone in 25 mg tablets was given orally in 3 cases, and cortisone acetate suspension (25 mg per ml) was given intramuscularly in 6 cases Adults received an initial dose of 200 mg. followed by 2 doses of 100 mg each at 6-hour intervals Children were given approximately two thirds the adult dose In all cases, the total duration of cortisone therapy did not exceed 12 hours Clinical improvement was uniformly observed within 24 hours or less after the initiation of combined therapy. The most striking observations were the prompt relief of headache and toxemia, and the return of appetite No deaths occurred The mean duration of fever after the start of combined [illegible]

[illegible] prevent the often severe residual cerebral and myocardial damage suffered by patients with Rocky Mountain spotted fever Harrell (1952) has also called attention to the necessary supportive measures, such as diet and good nursing care, that must be followed even with adequate antibiotic therapy in order to lessen the residual damages produced by the disease.

EPIDEMIOLOGY

Until 1930, spotted fever was believed to be confined to the northwest mountainous portions of the United States, although a case was reported in Indiana in 1925. At present, the disease has been reported from 46 states, the exceptions being Maine and Vermont (Dauer, 1957). It has also been recognized in Canada (British Columbia, Alberta and Saskatchewan), in Mexico (the northern part of the state of Sinaloa, the southern part of the state of Sonora, and parts of the states of Coahuila and Durango [Silva-Goytia and Elizondo, 1952]), in Brazil (São Paulo, Rio de Janeiro and Minas Gerais), in Panama (De Rodaniche and Rodaniche, 1950, Calero [illegible]

[illegible] *fiebre de Choix, fiebre pinta* and *fiebre manchada;* in Brazil it has been called exanthematic typhus of São Paulo or Minas Gerais typhus, whereas in Colombia it was originally designated Tobiã fever. For incidence of spotted fever in the western hemisphere see Tables 37 and 38.

In the western United States, most cases are reported in April, May and early June, the season of prevalence of the Rocky Mountain wood tick, *Dermacentor andersoni.* In sections of higher altitudes, such as Wyoming and Colorado, the danger period may extend into the summer. Occasional cases have been reported during the late summer, fall and even winter months. In the eastern United States, most cases occur during the summer, the season of the greatest activity of the American dog tick, *Dermacentor variabilis.* In the West, most cases occur in adult males since they, through vocational pursuits, are more exposed to tick-bites Persons living in live-stock range areas and particularly those handling sheep are in greatest danger. Other groups affected are forest service personnel, highway construction workers, railroad-section hands, prospectors, miners, trappers, hunters, fishermen, campers and tourists. Only a relatively small percentage of the cases occurs in city dwellers because of the fact that *D. andersoni* is generally found in the areas removed from habitation and ordinarily does not infest domestic animals On the other hand, in the East a high percentage of infections is among children and women [illegible] that [illegible] only

The virulence of the infection varies with the locality and is correlated in any selected area with the maximum level of virulence of the strain of rickettsia in the local tick population In the Bitter Root Valley of western Montana, the death rate for nonvaccinated

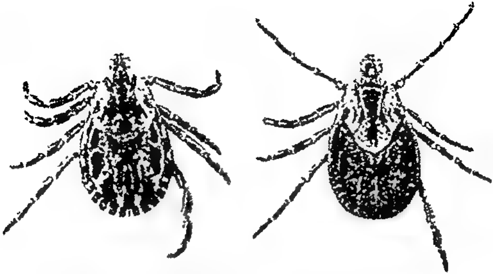

FIG 129 *Dermacentor variabilis* (*Left*) Male (*Right*) Female (Photograph from Dr R A Cooley, U S Public Health Service, Hamilton, Mont)

FIG 130 *Dermacentor andersoni* (*Left*) Male (*Right*) Female (Photograph from Dr R A Cooley, U S Public Health Service, Hamilton, Mont)

adults averages about 80 per cent and for children about 37 5 per cent A high case fatality rate also prevails in other parts of western Montana, in certain parts of Wyoming and Oregon, in all affected portions of northern Idaho, and along the extreme eastern edge of Washington In other areas of the West, notably southern Idaho, the case fatality rate varies with a minimum of at least 10 per cent (Parker, 1938) On the average, spotted fever in the East is about as fatal as it is in the West In comparing data for a 10-year period (1930-1939 inclusive), Topping (1941) reported that the crude fatality rate for Idaho and Montana was 28 1 per cent, while for Maryland and Virginia it was 18 4 per cent Little difference was found in the fatality rates when the two areas were compared on the basis of age In the western states, one half of the cases (50 2%) occurred in persons aged 40 years or over, while in the eastern states this was practically reversed with the largest number (46 8%) occurring in persons under 15 years of age As

already indicated, in the East, the incidence among females (39.4%) was considerably higher than in the West (16.5%). For additional information concerning the epidemiology of spotted fever in Maryland and Virginia, see Price (1954) and Cawley and Wheeler (1957), respectively.

The following brief outline of the distribution of known vectors and of those shown experimentally to be capable of transmitting spotted fever will serve to indicate the wide dispersal of ticks and the threat they represent to man (Steinhaus, 1946; Philip, 1942; Parker et al., 1933; Spencer and Parker, 1923, Davis, 1942, 1943a, b; Davis et al., 1941, Patiño-Camargo, 1941; Parker, 1938; Parker et al, 1943, Kohls, 1948) Within the United States, 4 species of ticks have been recognized as natural carriers: the Rocky Mountain wood tick, *D andersoni;* the Amer-

In Canada, the natural vector of spotted fever is *D andersoni*. In Mexico, naturally infected specimens of the common dog tick, *Rhipicephalus sanguineus*, have been reported found in the states of Sinaloa, Sonora, the lake region (Comarca Lagunera) of the states of Coahuila and Durango and in the states of San Luis Potosi (city of San Luis Potosi), Nuevo Léon (Montemorelos) and Michoacan (Morelia). Also, specimens of naturally infected *A. cajennense* have been reported in the city of Vera Cruz In addition to the above, Silva-Goytia and Elizondo (1952), in studies carried out in the Comarca Lagunera area, reported the isolation of spotted fever strains from *Ornithodorus nicollei* collected inside a room and on dogs, larvae of *Otobius lagophilus* collected on burros; and *R. sanguineus* collected on dogs and horses. These authors also reported the possible isolation of a spotted-fever strain of rickettsiae from human lice. They could not maintain the strain in guinea pig passage but claimed that it gave immunity to their experimental animals In both Colombia and Brazil, the principal natural vector is believed to be *A. cajennense*.

Ticks that may be placed in the category of potential carriers or that have been shown experimentally to be capable of transmission are· in the United States—the Cayenne tick, *Amblyomma cajennense;* the Pacific Coast tick, *D occidentalis;* the rabbit dermacentor, *D parumapertus;* the brown dog tick, *R. sanguineus; Ornithodorus parkeri; D. albipictus; O. hermsi; O. nicollei; O. turicata* and *O. rudis;* in Brazil—*A. striatum, A. brasiliense, R. sanguineus* and *O. rostratus;* in Colombia—*Anocentor nitens, O. rudis* and *R. sanguineus.*

Of all the ticks, *D. andersoni* has been studied most extensively in relation to spotted fever. It is found throughout the Rocky Mountain region and adjacent areas Rickettsiae may be found in all stages of the tick, from the egg through the adult; they may be transmitted during copulation, and infected females pass them to their progeny. So far as is known, the only way in which the rickettsiae survive the winter is in infected nymphs and adult ticks. Developmental forms of ticks feed on a great variety of small wild animals, principally rodents and rabbits, many of which are susceptible to spotted fever. Adult ticks mainly infect large wild and domestic animals but are also found on jack rabbits and porcupines The larvae are active during the summer, and the nymphs and the adults during spring and early summer. At higher altitudes, their activity may occur at later periods of the year The life cycle is normally completed in 2 years Adult ticks bite man readily, and occasionally nymphs have been found attached to children

D. variabilis is closely related, both morphologically and biologically, to *D. andersoni* (Cooley, 1938; Smith et al, 1946). It is found in the Great Plains region extending eastward to the Atlantic Coast and occurs sporadically in California and south-central Oregon To the south, it reaches into Mexico. In Canada, it occurs eastward from southern Manitoba and has even been reported in Labrador. Developmental forms feed on rodents, but certain species of mice are apparently more favored The dog is by far the preferred host of the adults, although they also feed on deer, cattle and other large, domestic or wild animals Nymphs engorge over a period of months and have been found feeding even during the winter Adult ticks appear in late spring and remain active longer during the summer than does *D andersoni* The importance of this tick lies in its close relationship with dogs and human habitations, the adult tick bites man readily.

Amblyomma americanum has been studied less intensively than either *D andersoni* or *D variabilis.* It is prevalent in the southern states around the Gulf Coast, occurs in southeastern and south-central parts of the United States and has been reported in Labrador and in South America as far down as Argentina. It feed

domestic animals and on certain species of birds. The immature stages, unlike those of *D. andersoni* and *D variabilis*, are commonly found on the same host animals as the adult ticks All stages of this tick attack man, and the larvae and the nymphs are notorious pests (Cooley and Kohls, 1944, Kohls, 1948)

Haemaphysalis leporis-palustris occurs throughout the United States, extending northward to central Alaska and the southern end of Hudson Bay and southward into South America This tick, in all stages, is largely restricted to the several species of wild rabbits and to certain ground-frequenting birds Several thousand ticks are sometimes found on a single host The importance of this tick to spotted fever does not depend on its direct connection with man, whom it rarely attacks, but upon the fact that by feeding on rabbits it supplies a possible source of rickettsiae for the *D andersoni*, *D variabilis* and *A americanum* feeding simultaneously on the the same host rabbit Thus, the rabbit tick, which spontaneously transmits spotted fever to rabbits, must be considered as a potent factor in the prevalence of spotted fever rickettsiae in the man-infesting species of ticks in the United States and Canada *H leporis-palustris* is prevalent in the northern United States from early spring to early fall, but in the South its period of activity is considerably extended Under favorable conditions, its life cycle is completed in 1 year *H leporis-palustris* consistently carries strains of spotted fever much milder or less virulent than those usually isolated from *D andersoni* (Parker, et al, 1951)

Rhipicephalus sanguineus is quite widespread but is especially prevalent in the warmer climates It is abundant in the Gulf Coast states and has been reported in Massachusetts, Ohio, Minnesota and even Nova Scotia It has been reported found naturally infected in the United States (Anigstein and Bader, 1943, and in Mexico (Bustamente et al, 1946) This species commonly attacks man in Europe and Africa, but in the United States there are few records of any forms hav-

animals as well as certain wild and domestic fowl It has been shown to be a vector of spotted fever in Brazil, Colombia and Mexico It is fortunate that its distribution within the United States is limited, since it is a vicious parasite of man in all its stages

Dermacentor occidentalis is found in California west of the Cascade Mountains and extends northward to central Oregon This tick, although limited in distribution, is nevertheless important because it could convey spotted fever to man and, in addition, will hybridize with *D. andersoni* (Cooley, 1938) The immature stages usually feed on rodents and other small animals, but they have also been found on cattle (Herms and Howell, 1936) The adults are found on larger animals such as deer, cattle and horses

Dermacentor parumapertus is found mainly in the southwestern areas of the United States which thus far are of minor importance in the epidemiology of spotted fever However, strains of rickettsiae related to the spotted fever group have recently been isolated from lots of the "rabbit dermacentor" tick collected in northern Nevada (Philip and Hughes, 1953, Philip et al, 1955) In the northern regions, its distribution overlaps that of *D andersoni* and in the West and the Northwest both that of *D. occidentalis* and *D variabilis* *D parumapertus* has been found, thus far, almost exclusively on rabbits, but occasionally it has been collected

taneously feeding

The winter tick, *Dermacentor albipictus*, has been strongly incriminated by circumstances in which a human case of Rocky Mountain spotted fever followed a tick-bite (Philip and Kohls, 1951) This is an interesting observation, since *D albipictus* rarely has been observed to attach itself to man even in areas where it is abundant on animals

Ornithodoros parkeri is found in the burrows and the nests of rodents in the northwestern states It has been shown to be an

fever rickettsiae in unfed adult ticks However, inoculation was apparently the most reliable method for testing ticks that had been fed recently No demonstrable infection resulted when infected, unfed ticks were inoculated into guinea pigs, but many of the

already indicated, in the East, the incidence among females (39 4%) was considerably higher than in the West (16 5%). For additional information concerning the epidemiology of spotted fever in Maryland and Virginia, see Price (1954) and Cawley and Wheeler (1957), respectively.

The following brief outline of the distribution of known vectors and of those shown experimentally to be capable of transmitting spotted fever will serve to indicate the wide dispersal of ticks and the threat they represent to man (Steinhaus, 1946, Philip, 1942, Parker et al., 1933, Spencer and Parker, 1923, Davis, 1942, 1943a, b, Davis et al., 1941; Patiño-Camargo, 1941; Parker, 1938, Parker et al, 1943; Kohls, 1948). Within the United States, 4 species of ticks have been recognized as natural carriers· the Rocky Mountain wood tick, *D andersoni*, the American dog tick, *D. variabilis*, the lone star tick, *Amblyomma americanum*, and the rabbit tick, *Haemaphysalis leporis-palustris*.

In Canada, the natural vector of spotted fever is *D andersoni* In Mexico, naturally infected specimens of the common dog tick, *Rhipicephalus sanguineus*, have been reported found in the states of Sinaloa, Sonora, the lake region (Comarca Lagunera) of the states of Coahuila and Durango and in the states of San Luis Potosi (city of San Luis Potosi), Nuevo Léon (Montemorelos) and Michoacan (Morelia). Also, specimens of naturally infected *A cajennense* have been reported in the city of Vera Cruz In addition to the above, Silva-Goytia and Elizondo (1952), in studies carried out in the Comarca Lagunera area, report

from

room

lus collected on burros; and *R. sanguineus* collected on dogs and horses. These authors also reported the possible isolation of a spotted-fever strain of rickettsiae from human lice They could not maintain the strain in guinea pig passage but claimed that it gave immunity to their experimental animals In both Colombia and Brazil, the principal natural vector is believed to be *A cajennense*

Ticks that may be placed in the category of potential carriers or that have been shown experimentally to be capable of transmission are: in the United States—the Cayenne tick, *Amblyomma cajennense;* the Pacific Coast tick, *D. occidentalis;* the rabbit dermacentor, *D. parumapertus;* the brown dog tick, *R. sanguineus; Ornithodorus parkeri; D. albipictus; O. hermsi; O. nicollei; O. turicata* and *O. rudis;* in Brazil—*A. striatum, A. brasiliense, R. sanguineus* and *O rostratus;* in Colombia—*Anocenter nitens, O. rudis* and *R. sanguineus.*

Of all the ticks, *D. andersoni* has been studied most extensively in relation to spotted fever. It is found throughout the Rocky Mountain region and adjacent areas. Rickettsiae may be found in all stages of the tick, from the egg through the adult; they may be transmitted during copulation, and infected females pass them to their progeny. So far as is known, the only way in which the rickettsiae survive the winter is in infected nymphs and adult ticks. Developmental forms of ticks feed on a great variety of small wild animals, principally rodents and rabbits, many of which are susceptible to spotted fever. Adult ticks mainly infect large wild and domestic animals but are also found on jack rabbits and porcupines The larvae are active during the summer, and the nymphs and the adults during spring and early summer. At higher altitudes, their activity may occur at later periods of the year. The life cycle is normally completed in 2 years Adult ticks bite man readily, and occasionally nymphs have been found attached to children

found in the Great Plains region extending eastward to the Atlantic Coast and occurs sporadically in California and south-central Oregon. To the south, it reaches into Mexico. In Canada, it occurs eastward from southern Manitoba and has even been reported in Labrador Developmental forms feed on rodents, but certain species of mice are apparently more favored. The dog is by far the preferred host of the adults, although they also feed on deer, cattle and other large, domestic or wild animals. Nymphs engorge over a period of months and have been found feeding even during the winter. Adult ticks appear in late spring and remain active longer during the summer than does *D. andersoni* The importance of this tick lies in its close relationship with dogs and human habitations; the adult tick bites man readily.

Amblyomma americanum has been studied less intensively than either *D. andersoni* or *D variabilis*. It is prevalent in the southern states around the Gulf Coast, occurs in southeastern and south-central parts of the United States and has been reported in Labrador and in South America as far down as Argentina. It feeds on a variety of wild and

domestic animals and on certain species of birds The immature stages, unlike those of *D andersoni* and *D. variabilis*, are commonly found on the same host animals as the adult ticks All stages of this tick attack man, and the larvae and the nymphs are notorious pests (Cooley and Kohls, 1944, Kohls, 1948)

Haemaphysalis leporis-palustris occurs throughout the United States, extending northward to central Alaska and the southern end of Hudson Bay and southward into South America. This tick, in all stages, is largely restricted to the several species of wild rabbits and to certain ground-frequenting birds Several thousand ticks are sometimes found on a single host The importance of this tick to spotted fever does not depend on its direct connection with man, whom it rarely attacks, but upon the fact that by feeding on rabbits it supplies a possible source of rickettsiae for the *D andersoni*, *D variabilis* and *A americanum* feeding simultaneously on the the same host rabbit Thus, the rabbit tick, which spontaneously transmits spotted fever to rabbits, must be considered as a potent factor in the prevalence of spotted fever rickettsiae in the man-infesting species of ticks in the United States and Canada *H leporis-palustris* is prevalent in the northern United States from early spring to early fall, but in the South its period of activity is considerably extended Under favorable conditions, its life cycle is completed in 1 year *H. leporis-palustris* consistently carries strains of spotted fever much milder or less virulent than those usually isolated from *D andersoni* (Parker, et al , 1951)

Rhipicephalus sanguineus is quite widespread but is especially prevalent in the warmer climates It is abundant in the Gulf Coast states and has been reported in Massachusetts, Ohio, Minnesota and even Nova Scotia. It has been reported found naturally infected in the United States (Anigstein and Bader, 1943, and in Mexico (Bustamente et al., 1946) This species commonly attacks man in Europe and Africa, but in the United States there are few records of any forms having been found on hosts other than the dog, which is the principal host of all stages

Amblyomma cajennense is found in southern Texas and Florida and is abundant in Mexico, Central America and parts of South America It parasitizes a variety of wild and domestic animals as well as certain wild and domestic fowl It has been shown to be a vector of spotted fever in Brazil, Colombia and Mexico It is fortunate that its distribution within the United States is limited, since it is a vicious parasite of man in all its stages

Dermacentor occidentalis is found in California west of the Cascade Mountains and extends northward to central Oregon This tick, although limited in distribution, is nevertheless important because it could convey spotted fever to man and, in addition, will hybridize with *D. andersoni* (Cooley, 1938) The immature stages usually feed on rodents and other small animals, but they have also been found on cattle (Herms and Howell, 1936) The adults are found on larger animals such as deer, cattle and horses

Dermacentor parumapertus is found mainly in the southwestern areas of the United States which thus far are of minor importance in the epidemiology of spotted fever However, strains of rickettsiae related to the spotted fever group have recently been isolated from lots of the "rabbit dermacentor" tick collected in northern Nevada (Philip and Hughes, 1953, Philip et al , 1955) In the northern regions, its distribution overlaps that of *D andersoni* and in the West and the Northwest both that of *D occidentalis* and *D variabilis* *D parumapertus* has been found, thus far, almost exclusively on rabbits but occasionally it has been collected on deer, coyote and man Its chief importance would be its conveyance of spotted fever to rabbits on which developmental forms of other known vectors of the disease were simultaneously feeding

The winter tick, *Dermacentor albipictus*, has been strongly incriminated by circumstances in which a human case of Rocky Mountain spotted fever followed a tick-bite (Philip and Kohls, 1951) This is an interesting observation, since *D albipictus* rarely has been observed to attach itself to man even in areas where it is abundant on animals

Ornithodoros parkeri is found in the burrows and the nests of rodents in the northwestern states It has been shown to be an efficient vector of spotted fever (Davis, 1942) It rarely bites man spontaneously, but it will do so readily in the laboratory

Spencer and Parker (1923) reported that it was unwise to rely upon feeding or inoculation alone as an index of the presence of spotted fever rickettsiae in unfed adult ticks However, inoculation was apparently the most reliable method for testing ticks that had been fed recently No demonstrable infection resulted when infected, unfed ticks were inoculated into guinea pigs, but many of the

animals were found subsequently to be immune to spotted fever. The transition of the rickettsiae from a nonvirulent, immunizing phase to a virulent, infection-producing phase, brought about by the ingestion of fresh blood was called "reactivation." As Spencer and Parker (1923) pointed out, "It is not known whether this transition is due to multiplication of the virus, to development of a possible distinct stage in its life cycle, to renewal of virulence following a period of attenuation, or perhaps some other unrecognized condition initiated by the ingestion of fresh mammalian blood." The "reactivation" phenomenon probably explains why ticks do not infect unless they have been attached and have fed for several hours. Ricketts, 1907a, observed an immunizing phase of rickettsiae in tick eggs Spotted-fever infection was produced by the injection of from 5 to 80 eggs recently laid by an infected female tick However, immunity instead of frank infection was produced with eggs that had been dried for 4 months Spencer and Parker (1923) likewise produced immunity in guinea pigs by injecting them with comparatively fresh eggs from an infected rabbit tick, *H leporis-palustris*.

Spencer and Parker (1930) showed that tick rickettsiae would produce infection of guinea pigs through the unabraded skin and uninjured conjunctival sacs, and they suggested that infection of man in this way is a distinct possibility and doubtless occurs occasionally Infection in such a manner could occur if an infected tick were crushed between the fingers when handpicking ticks from domestic animals or when handling tick-infested small animals (rabbits, ground squirrels, etc.) that had been trapped or shot. Fresh tick feces are also infectious, but the rickettsiae in feces are much less virulent than those in tick tissue and apparently do not infect through unabraded skin However, both tick tissues and feces can produce infection through an abrasion. Dried tick feces rapidly lose their infectiousness, so that infection via the respiratory tract by dry, airborne feces, as happens with louse feces in epidemic typhus, is not likely.

Control Measures

There are 3 possible ways of preventing spotted-fever infection (1) personal care, (2) vaccination and (3) use of tick-control methods.

Under personal care are included avoidance of tick-infested areas, use of tick repellents, the wearing of suitable clothing so as to minimize the possibility of tick-bites, and the early removal of ticks that may become attached to the body. Known infested areas should be avoided as much as possible during the tick season; however, it is recognized that this is not possible for many persons living in affected areas. Furthermore, any area in which a tick vector is present is potentially dangerous, and the areas in which the disease is known to exist are constantly expanding Certain precautions should be taken by persons entering tick-infested areas. Camp sites should not be located where small animals are numerous, since ticks would be found more frequently in such areas A tick-repellent, such as N-butylacetanilide solution should be distributed uniformly throughout the outer garments It is important to wear proper clothing (the shirt should be tucked inside the trousers, and high boots, leggings or socks should be worn outside the trouser legs) so that ticks will find it more difficult to become attached

If one spends much time in tick-infested country, some ticks will reach the body in spite of all precautions. However, since ticks seldom attach themselves to the skin immediately, and rarely transmit infection until they have fed for several hours, an effective method of prevention is to remove the clothing and search both the clothing and the body for ticks at least twice each day Attached ticks should be removed immediately by grasping the body of the tick with small forceps, gloved fingers or with a piece of paper held between the fingers, and pulling gently. Bare fingers should not be used because of the danger of contamination with rickettsiae Twisting the tick or pulling it forcibly may cause it to break and leave the mouthparts in the skin. The wound itself should be treated with an antiseptic solution, such as tincture of iodine There is nothing characteristic which will distinguish the bite of an infected tick from that of a noninfected one

Vaccination is an effective method of prophylaxis Two types of vaccine are available A preparation made from the tissues of infected ticks (*D. andersoni*) is prepared and distributed by the Rocky Mountain Laboratory, United States Public Health Service,

Hamilton, Mont. A second type of vaccine, prepared from rickettsiae grown in the yolk-sac tissue of fertile hen's eggs (Cox, 1941; 1948), is produced by certain commercial manufacturers of biologics in the United States.

All evidence from animal experiments, as well as from use in human beings, indicates that the yolk-sac and the tick vaccines stimulate the same degree of immunity It is recommended that these vaccines be given either subcutaneously or intramuscularly in 3 injections of 1 0 ml each, or in 2 injections of 2 0 ml each, at 5- to 7-day intervals They should be administered in the winter or early spring, before the advent of the tick season, and should be repeated each year, since the maximum degree of protection is retained for less than a year. Those individuals sensitive to chick or egg proteins should be given the tick-tissue vaccine

The vaccines have definite protective value The degree and the duration of immunity vary with the individual vaccinated and with the virulence of regional strains of spotted fever The vaccine usually affords complete protection against relatively mild strains but may be less effective against more virulent ones Most children are fully protected against even the highly fatal types of spotted fever, whereas adults are fully protected only occasionally However, in the latter, the degree of protection is sufficient to modify the severity of the disease and to ensure recovery in practically all cases It is questionable that the vaccine is of value after infection has been acquired, it is of no value in treatment after onset of illness In 1941, Cox reported on the development of a living, avirulent strain of spotted fever rickettsiae derived from *D variabilis* by continued passage in the yolk sac of fertile hens' eggs and suggested that it might be of value for immunization of human beings, but no practical follow up has been made thus far.

With the advent of the antibiotics, some persons believe that there is now no need for vaccination However, persons who are exposed to more than ordinary risk should be vaccinated It is well known that spotted fever has proved to be fatal in unvaccinated persons who have been treated with antibiotics late in the course of the disease Thus, preventive as well as therapeutic measures should be practiced where the incidence of spotted fever is high.

During the past few years, it has been shown that roadside and pathside control of *D. variabilis* and *A americanum* is so readily obtained with insecticides that chemical control is of practical value in inhabited areas where spotted fever is a serious hazard or even where tick annoyance only is a problem Smith et al (1946) pointed out that the adults of the American dog tick (*D. variabilis*) tend to concentrate on the grass at the sides of the roads and paths They reported that a spray containing 0 5 per cent DDT (2,2 bis [p-chlorophenol]-1,1,1-trichlorethane), 2.5 per cent of soluble pine oil and 97 per cent water, applied to roadside vegetation at the rate of 7 pounds of DDT per acre, was not injurious to foliage and reduced tick abundance to less than 1 per cent of the original infestation for 10 days, whereas tick abundance in an untreated area, ranged from 82 to 106 per cent during the same time. Glasgow and Collins (1948) reported that DDT applied at the rate of 1 pound per acre, in the form of dusts, solutions, emulsions or suspensions, gave excellent control at a lower cost for materials than any other acaricide tested Benzene hexachloride used at the rate of 1 pound of the gamma isomer per acre apparently gave equally good results but cost more

Therrien et al (1954) found dieldrin applied as a dust at 1 pound per acre was more effective in controlling *A americanum* than any of the other chemicals tested McCroan et al (1955) likewise reported good results in the control of ticks by chemical methods They used widestrip spraying equipment in which mist generators gave excellent coverage of a 50- to 100-foot strip along roadways They state that "if rosin-DDT emulsion is used in a good mist machine, 1 5 pounds of DDT per acre gives good residual control of *D variabilis* for an entire tick season" The rosin-DDT emulsion did not burn the vegetation when applied along roadsides

TICK-BORNE TYPHUS

(Synonyms boutonneuse fever, *fièvre boutonneuse*, Kenya typhus, South African tick bite fever, *fièvre escharonodulaire*, ex-

animals were found subsequently to be immune to spotted fever. The transition of the rickettsiae from a nonvirulent, immunizing phase to a virulent, infection-producing phase, brought about by the ingestion of fresh blood was called "reactivation" As Spencer and Parker (1923) pointed out, "It is not known whether this transition is due to multiplication of the virus, to development of a possible distinct stage in its life cycle, to renewal of virulence following a period of attenuation, or perhaps some other unrecognized condition initiated by the ingestion of fresh mammalian blood" The "reactivation" phenomenon probably explains why ticks do not infect unless they have been attached and have fed for several hours Ricketts, 1907a, observed an immunizing phase of rickettsiae in tick eggs Spotted-fever infection was produced by the injection of from 5 to 80 eggs recently laid by an infected female tick However, immunity instead of frank infection was produced with eggs that had been dried for 4 months Spencer and Parker (1923) likewise produced immunity in guinea pigs by injecting them with comparatively fresh eggs from an infected rabbit tick, *H leporis-palustris*.

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Pathologic Picture

In man the microscopic pathologic lesions are characteristic of the rickettsial diseases and consist of thrombosis of the capillaries and the small arteries and veins, resulting from lesions of the vascular endothelium. The thrombosis is associated with the development of perivascular nodules In guinea pigs, the pathology duplicates in all essential details that of spotted fever (Hass and Pinkerton, 1936). Intraperitoneal inoculation of adult male guinea pigs with infected blood from a patient causes a high fever, following an incubation period of 4 to 7 days The reac-

sacrificed at the height of fever (2nd or 3rd day) show the spleen to be 2 or 3 times larger than normal, the testes are injected, and the tunicae may be adherent to the scrotal sac Frequently, the testicles and the spleen are covered with a whitish exudate In the infected cells, rickettsiae may be seen both intra- and extranuclear Inoculated rats and gerbils show only inapparent infections

Experimental Infection, Host Range

Durand (1932a) produced the disease experimentally in 13 human beings in 3 by inoculation of triturated infected ticks, in 7 by inoculation of blood from naturally infected patients, in 2 by inoculation of brain suspensions from animals inoculated with blood from naturally infected patients, and in 1 by inoculation of a *tâche noire* taken at biopsy Blanc and Caminopétros (1932) demonstrated that the disease could be reproduced experimentally in human beings by subcutaneous inoculation or by application of macerated infected tick tissue to slightly traumatized conjunctivae In North Africa, and more generally in the Mediterranean basin, dogs undoubtedly play an important role and they, particularly pups, probably constitute the chief animal reservoir Blanc and Caminopétros (1932) were unsuccessful in their attempts to infect dogs either with the blood of patients in the stage of full eruption or with proved infected ticks, but Durand (1932b) succeeded in showing their susceptibility Blanc and Caminopétros (1932) have shown that white mice may have inapparent infections, and that the Macedonian spermophile (*Citellus citellus*) is highly susceptible but because of its limited habitat could not be considered as a universal reservoir of the rickettsiae Durand and Laigret (1932) extended the studies to wild rodents of France and Africa which, because of their association with human habitations, could be considered as potential hosts of the vector *Mus barbarus*, *Jaculus jaculus* and *Meriones shawi*, found frequently around Tunis, were fully resistant to infection. Inapparent infections were obtained with 2 white rats (*Mus rattus*), while from the gerbil (*Gerbillus campestris*) they succeeded in passing the infection to man by inoculating a mixture of blood and brain tissue from an infected animal. Blanc and Caminopétros (1932) suggested that a vertebrate host is not essential for maintenance of the rickettsiae, and that ticks can maintain the cycle not only because they remain infectious after hibernation but also because they transmit the infection through their eggs In South Africa, it appears that tick-borne typhus is not necessarily related to dogs, for infection is transmitted by ticks which are veld dwellers and not found on domestic animals, Gear, 1938 However, Alexander and Mason (1939) reported the isolation of a strain from a tick-infested dog that became ill More recently, Gear (1954) reported the isolation of a strain of rickettsiae

also showed a positive complement-fixation test for tick-borne typhus and, judging by the results of complement-fixation tests, it would appear that a considerable number of rats in the suburban areas of Johannesburg are infected However, it was believed that rats, like man, were infected by ticks picked up

irroratus) showed positive reactions with *R conorii*, thus indicating past infection with this rickettsia This lead was followed up, and a strain of tick-borne typhus was isolated from the brain of the vlei rat For a good chart depicting the present concept of the natural history of *R conorii*, as it occurs in South Africa at least, see Gear (1954)

anthème typhoïde estival, dothiendermie aigue, exanthème infectieux épidémique, exanthematous fever, eruptive fever, Marseilles fever, fever of Conor and Bruch)

Introduction

Tick-borne typhus is a tick-borne, typhus-like, febrile disease, generally mild in nature, often characterized by a black spot, *tâche noire*, at the site of tick-bite, swelling of the corresponding lymph nodes, and a maculopapular eruption that covers the entire body, including the palms of the hands and the soles of the feet.

History

Conor and Bruch first described the disease as a clinical entity in Tunis, North Africa in 1910. In 1911, Nuttall gave the name "tick-bite fever" to a similar disease described independently by McNaught, 1911, and Sant' Anna, 1911. Comparative studies carried out by Pijper, 1936, and Pijper and Dau, 1934, on spotted fever, boutonneuse fever and tick-bite fever led them to the conclusion, Pijper, 1936, that "tick-bite fever must be regarded as a rickettsiosis *sui generis* and that it deserves a place by itself in the group" However, the studies of Mason and Alexander, 1939a, b; Gear and Bevan, 1936, Gear and Douthwaite, 1938, Gear, 1938, Gear and De Meillon, 1939, and Gear (1950) showed that there was complete cross immunity between tick-bite fever of South Africa and boutonneuse fever of North Africa

All present evidence indicates that the boutonneuse fever infections that have been reported from practically all of the Mediterranean countries of Europe, Asia and Africa (cf. Olmer and Olmer, 1956) and the other tick-borne rickettsioses found throughout Africa, such as Kenya typhus, Nigerian typhus, tick typhus of Abyssinia and South African tick-bite fever, should be grouped together under the general terminology of tick-borne typhus, of which *R conorii* is the causative agent (Joint OIHP/WHO Study Group on African Rickettsioses, 1950)

Clinical Picture

The incubation period generally is from 5 to 7 days, although occasionally it may be [illegible] from 8 to 14 days with defervescence taking place by rapid lysis Associated symptoms are a violent and persistent headache, commonly frontal, but which may be retro-orbital or general; a feeling of lassitude, and pains in various joints and muscles Photophobia is often present. Bulbar conjunctivae may be hyperemic and slightly edematous. The eyeballs may be sore. There may be mental confusion and delirium The tongue is usually coated, peeling at the sides and the tip. Frequently (in about 80% of cases) at the time of onset a small ulcer (black spot, *tâche noire*) from about 2 to 5 mm in diameter, showing a black necrotic center surrounded by a dark reddish area of variable dimensions, appears at the site of the tick-bite and may persist until the temperature falls. The *tâches noires* may be found on any part of the body, usually on those covered by clothing, and invariably are accompanied by enlargement and tenderness of the regional lymph nodes. Usually 3 or 4 days after the initial chill, a maculopapular eruption develops. The rash is usually first noticed on the forearms as discrete rose-colored elevated spots The rash spreads rapidly over the whole body involving the palms, the soles and even the face. It increases in density over the next few days. The rash, in severe cases, may become hemorrhagic, especially on the lower limbs The rash on the abdomen may be less pronounced than elsewhere Small, round, red spots which persist only a few days may be found on the soft palate The macules, which at first disappear on pressure, are small, clearly outlined and separated by healthy skin They soon become papular, are occasionally hemorrhagic and may persist for some time after defervescence. The eruption disappears without leaving residual traces and there is no desquamation During the febrile stage the patient often shows mental excitement giving way to a very marked depression during convalescence. Marked weakness is characteristic of convalescence with a tendency to tachycardia on exertion The prognosis is usually good However, the disease is not always mild, since both Gear, 1938, and Dezest (1953) have described grave forms with complications or death in aged or physically deficient persons For a good clinical picture of tick-borne typhus in Kenya, East Africa, see Harris (1952).

most certainly tick-borne typhus The reaction with Proteus OXK apparently is even more frequent, and the titers on the average are higher than with OX2 or OX19 (Joint OIHP/WHO Study Group on African Rickettsioses, 1950) The agglutination titers with Proteus organisms are generally low, varying between 1 50 and 1 500 The lowest reliable, suggestive titer is 1 50 However, with the use of the Weil-Felix test alone, it is not possible to differentiate tick-borne typhus from epidemic (louse) or murine (flea-borne) typhus The need for a more specific test has been met by the use of specific rickettsial agglutination and complement-fixation tests. These tests, together with the isolation of the rickettsiae by cultural methods, accurately distinguish tick-borne typhus from either epidemic or murine typhus With washed, purified rickettsial antigens prepared from infected yolk sacs, Plotz et al (1944) showed that tick-borne typhus could be readily differentiated from spotted fever by the complement-fixation reaction However, it must be remembered that a disadvantage of these more specific rickettsial tests is that antibodies may be detectable for many years after the patient's illness, and thus they do not differentiate recent from long previous infection However, the change from a negative to a positive result is of diagnostic value

Treatment

Chlortetracycline has been reported to be a specific treatment for tick-borne typhus (Gear and Harington, 1949, Hildick-Smith, 1949, Yeo, 1950, Harris, 1952, 1953) Following chlortetracycline therapy, normal temperatures were attained in 48 to 72 hours and there were no relapses Chlortetracycline given at the time of the appearance of the primary lesions will abort the febrile attack Choramphenicol likewise has been found to be efficacious, and undoubtedly oxytetracycline would give satisfactory results The treatment schedule for spotted fever is recommended

Epidemiology

Following the description of cases in Tunis in 1910, the disease was reported in France (the Avignon region), Tripoli, Italy, Greece, Crete and Rumania (Blanc and Caminopétros, 1932) Olmer and Olmer (1956) have reviewed rather comprehensively the epidemi-

1933 were the departments of Bouches-du-Rhône, Var, Alpes-Maritimes, Vaucluse, Gard and Herault. Three more recent important foci are Lyon, Toulouse and the department of Landes The disease is found throughout Italy and is not rare in Sicily It has been reported in Spain, Greece, Crete, Turkey,

most common vector is the dog tick *Rhipicephalus sanguineus* Apparently, the disease has not been reported thus far from Jugoslavia and Albania (Olmer and Olmer, 1956) The disease is also widespread throughout all other parts of Africa such as Somaliland, Kenya, Uganda, Nigeria, the Sudan, Tanganyika, Nyasaland, Ghana (the Gold Coast), Sierra Leone, French Guinea, Senegal, Togoland, the Cameroons, Oubangui-Chari, the Belgian Congo, Ruanda-Urundi, the Portuguese colonies of Angola and Mozambique, and all parts of the Union of South Africa In Kenya,

cidence with most cases occurring in the summer months when ticks are numerous In the lowveld where the temperature is more uni-

not been isolated from this species Gear (1954) states, "It seems probable that most species of ixodid ticks are capable of transmitting the disease" It has been demonstrated that all stages of the common dog tick *Haemaphysalis leachi*—larva, nymph and adult—are infective and that there is hereditary transmission of the rickettsiae through

While the monkey is susceptible, the guinea pig is probably the most useful animal for experimental purposes. Tick-borne typhus may be transmitted to them by intraperitoneal injection of patient's blood. Blood taken during the first 5 days of illness or from patients who shortly develop a rash is best for making isolations. Guinea pigs inoculated intraperitoneally show an incubation period of 3 to 6 days, followed by a rise in temperature to 104° to 105° F. for 4 to 6 days, after which the temperature gradually returns to normal. Marked scrotal swelling with the tunicae adherent to the scrotal sac develops, but generally no sloughing results. Transfer of the infection is accomplished most readily by injecting guinea pigs intraperitoneally with testicular and tunica washings or with brain tissue taken from an animal at the height of fever; the minimum infective dose is about 0.003 Gm. Jadin (1951) reported infecting mice by inoculating them intranasally with specimens of ticks (*Haemaphysalis leachi*) taken from a Pekinese dog in Kabgaye, Belgian Congo. The lungs of the mice were filled with rickettsiae, but all attempts to maintain the strain by serial passage in mice failed.

Etiology

The etiologic agent is *R. conorii* (Joint OIHP/WHO Study on African Rickettsioses, 1950)—*Dermacentroxenus rickettsi*, var. *pijperi*, Mason and Alexander, 1939b, named after A. Conor, who, together with A. Bruch, published in 1910 the first clinical description of tick-borne typhus fever (boutonneuse fever) cases occurring in Tunis, North Africa. *R. conorii* is morphologically and tinctorially similar to *R. rickettsii*. As stated previously, all present evidence indicates that *R. conorii* is the etiologic agent of all the tick-borne rickettsial fevers of the Mediterranean countries of Europe, Asia and Africa as well as those found throughout Africa. Ticks, in which the etiologic agent *R. conorii* is hereditary, form the essential reservoir of infection. The rickettsiae live for more than 18 months in infected ticks without producing apparent deleterious effects in their hosts (Brumpt, 1932). Hass and Pinkerton (1936) found rickettsiae in nearly all tissues of infected ticks, particularly in the cells of the gut, the hypoderm and the ovaries where they apparently occur more abundantly shortly after feeding. A few rickettsiae may be found in the testicular exudate of infected guinea pigs, and occasionally in smears from the spleen, but are not found in smears made from the liver, adrenals, lungs, meninges or kidneys. Infected cells may show both intracytoplasmic and intranuclear rickettsiae, although rickettsiae are more often found in the cytoplasm. Like other rickettsiae, they stain well by the Giemsa, Castaneda or Macchiavello methods. The rickettsiae have not been cultivated on artificial media, but they grow readily in plasma-clot, agar-slant or Maitland-type tissue cultures and also in the yolk sac of the developing chick embryo (Cox, 1938). Alexander and Mason (1939) reported that *R. conorii* multiply intranuclearly when grown on the chorio-allantoic membrane of the fertile hen's egg. Intranuclear rickettsiae also are readily found in plasma-clot and agar-slant tissue cultures. Blanc and Caminopétros (1932) reported that *R. conorii* pass L_2 Chamberland candles and claimed that the virulence of human blood is lost after 12 days in an icebox. All attempts to pass *R. conorii* through Berkefeld candles or Seitz filter pads have failed. Guinea pigs recovered from Rocky Mountain spotted fever are solidly immune to tick-borne typhus, and vice versa. However, killed spotted-fever vaccines made from infected *D. andersoni*, which afford complete protection against Rocky Mountain spotted fever in guinea pigs, give no protection against tick-borne typhus (Davis and Parker, 1934; Hass and Pinkerton, 1936).

Diagnosis

In tick-borne typhus, positive serologic reactions appear late in the course of the disease, are rarely obtained before the 10th day of illness, and the maximum titer is usually attained 2 to 4 weeks after defervescence. Gear (1954) states that the average of a large series of cases indicates that Proteus OX19 and Proteus OX2 are agglutinated to

agglutinins may be present in higher titer. A higher agglutination with OX2 than with OX19 indicates the probability of tick-borne typhus; agglutination with OX2 only is al-

been reported to have caused 4 deaths in 12 cases (Le Gac et al., 1952; Le Gac, 1953). An interesting observation is that in the United States the primary lesion (eschar) always heals without leaving a scar, whereas in Africa the primary lesion is much more marked, is followed by considerable necrosis and destruction and always leaves a permanent scar.

Pathologic Picture

The gross appearance of the primary lesion of rickettsialpox is quite similar to the initial lesions seen in scrub typhus and tick-borne typhus. According to Dolgopol (1948), the *microscopic* picture of the primary lesion of rickettsialpox closely resembles the histologic findings of the initial lesions of scrub typhus as described by Allen and Spitz (1945). "The pustules in these two diseases are identical. The difference in the inflammation of the corium is largely quantitative. The area of polymorphonuclear infiltration of the corium in rickettsialpox is more limited and more superficial than in scrub typhus, and there is no degeneration of the connective tissue. The vascular changes also are less severe. The cellular infiltrates follow the same perivascular and periadnexal character as in scrub typhus, but the plasma cells are absent and the mast cells are more numerous. The vesicle of the rash is unique in a rickettsial disease. The epithelium at the top of the vesicle shows vacuolization and some disintegration of the cells with karyorrhexis. The basal epithelium is largely intact. The corium immediately beneath the vesicle shows some migration of polymorphonuclear cells and a slight diffuse mononuclear infiltration, but in the papular portion of the eruption which forms the base of the vesicle the changes in the corium are the same as in other papular lesions."

In albino mice, intraperitoneal inoculation of infectious material results in definite signs of illness, and death frequently occurs. Mice that die or that are sacrificed when moribund show a small amount of blood-tinged peritoneal fluid, enlarged lymph nodes, an enlarged, edematous liver and a dark, engorged spleen 2 to 10 times normal size. The respiratory and intestinal tracts show no gross changes. Male guinea pigs inoculated intraperitoneally with tunica washings or infected yolk-sac tissue show redness and swelling of the scrotum with adherence of the testes to the tunica vaginalis which is thickened and markedly injected, moderately enlarged spleen and lymph nodes, occasional small areas of pneumonic consolidation and indurated cutaneous and subcutaneous nodules at the site of inoculation.

Experimental Infection, Host Range

Wild house mice (*Mus musculus*) trapped in nonendemic areas, guinea pigs, chick embryos and albino mice are susceptible. Mice inoculated intraperitoneally show ruffled fur as early as the 6th day after inoculation. Either brain or spleen tissue may be used for transfer. The peak of the disease is generally reached between the 9th and the 13th days, and death may occur at any time during this period. Male guinea pigs inoculated intraperitoneally with tunica washings first show a scrotal reaction on about the 5th day. Onset of fever may occur from the 4th to the 6th day. A febrile period, marked by remission, lasts from 3 to 5 days. Guinea pig blood is not as infectious as tunica washings and gives less consistent results on inoculation. Male guinea pigs inoculated intraperitoneally with 10 per cent yolk-sac suspensions show a short incubation period (1 or 2 days) followed by a rapid onset of high fever which is sustained without remissions for 4 or 5 days. The scrotal reaction is usually delayed until the 4th day. Chick embryos are highly susceptible to infection and show large numbers of rickettsiae both in the yolk-sac tissue and the amniotic sac. Infected yolk-sac tissue diluted 1:10 to 1:10,000 produces death of embryos in 4 to 7 days. Attempts to produce the disease in monkeys even with massive doses of infected yolk-sac suspensions have failed.

Etiology

The etiologic agent has been classified with the rickettsiae, and the name *Rickettsia akari* (Greek, mite) was proposed by Huebner et al. (1946). The organism stains poorly with methylene blue or by Gram's method but stains well with Giemsa or by Macchiavello's method, and the red-staining diplobacillary and diplococcal forms closely resemble *R. prowazekii* and *R. typhi*. *R. akari* grows in the nucleus as well as in the cytoplasm of infected yolk-sac cells—in common with the rickettsiae of the spotted fever group. During the early studies of Huebner et al., 2 strains were isolated from the blood of patients, 6

the egg to succeeding generations The hereditary transmission may continue indefinitely. Hereditary transmission probably also occurs in other species of ixodid ticks Thus no mammalian reservoir may be necessary for maintaining the infection in nature However, it is believed that wild rodents may help in disseminating the infection It has been shown experimentally that all species of ticks found in the Union of South Africa can be infected (Gear, 1950)

Control Measures

No vaccine is available at present The preventive measures are those recommended for spotted fever. avoidance as far as possible of tick-infested areas and use of all precautions to prevent tick bite Since dogs especially are readily infested with *R sanguineus*, it is advisable to free them of ticks frequently

RICKETTSIALPOX

(Synonyms—*Rickettsiose vesiculeuse* (vesicular rickettsiosis), French literature, *Gamaso-Rickettsiosis Vesiculosa* (vesicular rickettsiosis), Russian literature)

Introduction

Rickettsialpox is usually a mild febrile disease, generally characterized by an initial lesion caused by the bite of an infected mite.

History

Rickettsialpox is the name given by Huebner et al (1946) to a newly recognized rickettsial disease first reported in New York City and described independently by Sussman (1946), Shankman (1946) and Greenberg et al. (1947). Because of its clinical similarity to chickenpox, it was named rickettsialpox

Clinical Picture

The illness is characterized by an abrupt onset of chills, fever, sweats and backache, generally followed 3 or 4 days later by a rash. Usually about a week prior to the onset of fever, a firm, red papule appears at the site of a mite-bite The lesion develops into a deep-seated vesicle which ultimately shrinks and dries to form a black eschar The initial lesion persists approximately 3 or 4 weeks and, in a fully developed state, frequently resembles certain stages of a vaccinia vesicle The primary lesion (or eschar) is found chiefly on the covered parts of the body, although it may occur on the neck, the face, the arms and the dorsum of the hands. However, a primary lesion apparently has not been observed in every case Thus, it was absent in 1 of 12 cases reported by Barker (1949), in 4 of 86 cases studied by Greenberg et al. (1947), and in 6 of 35 patients observed by Rose (1949)

Rash appears in all cases and is noticed either at the onset of fever or several days later At first the lesions are maculopapular, discrete and erythematous, but after a day or so vesicles develop in the summit of the papules. They dry, forming black crusts which ultimately fall off without producing scars The rash may be scanty, moderate or abundant There is no pattern in its distribution, and it may appear first on the arms, the legs, the abdomen, the back, the face or the chest As a rule, it has not been observed on the palms and the soles, although LaBoccetta et al. (1952) have reported a suspected case of rickettsialpox in a 14-year-old white boy who showed a rash over the chest, the abdomen, the back and the extremities, including the palms and the soles. The duration of the rash varies from 2 or 3 days in mild cases to 10 days in the most severe.

Except for fever and rash, there are no unusual signs. Fever with morning remissions is often low grade at onset but usually rises rapidly to 103° or 104° F. and persists for about a week. The temperature gradually returns to normal The regional lymph nodes usually become enlarged and tender An enlarged spleen occurs in a few cases, general lymphadenopathy is uncommon Red blood cell counts and the amount of hemoglobin are normal There is a moderate leukopenia with white cells varying between 2,400 and 7,500 per cu mm. The leukopenia usually lasts only during the acute illness and disappears about 2 weeks after onset Severe headache with frontal and retro-orbital pain occurs in practically all cases Backache and general muscular soreness are common early in the disease, lassitude is always present; and photophobia is not an infrequent symptom In the United States, rickettsialpox thus far has been considered to be a mild disease, but in French equatorial Africa (Oubangui-Chari) it has

as the *vector* of the disease to man. It may

distinct stages egg, larva, protonymph, deutonymph and adult male and female The larva does not feed Blood meals, which are taken by both nymphal stages and by adults of both sexes, afford many opportunities for transmission of a pathogen during feeding by a single mite. The adult stages successfully withstand starvation for longer periods of time than do the protonymphs. As early as 1942, Ewing reported that the mite fed on human beings.

The disease has been reported to occur in other countries as well as the United States in French equatorial Africa (Oubangui-Chari) (LeGac et al 1952, LeGac, 1953); in the Soviet Union (Zdrodovskii and Golinevich, 1953; 1956b, Scientific Session Summaries, 1954) On the basis of clinical and serologic evidence, it is believed to occur in South Africa (Gear, 1954)

In French Africa, the disease has not been seen in Europeans, but only in Negroes Attempts to isolate rickettsiae from insect parasites (*Leolaps nuttali*) collected from mice (*Mus musculus*) taken in the mud walls of the huts ended in failure Likewise, no strains of *R. akari* were actually isolated from patients who were ill, but the serum of a single guinea pig inoculated with blood from one patient showed agglutinins against an American strain of *R akari* in a titer of 1 40 on the 40th day after inoculation

Within the past few years (1946-1950)

ettsiosis" (*Gamaso-rickettsiosis vesiculosa*) (Scientific Session Summaries, 1954) The disease is stated to be transmitted by several species of "gamasid" mites (*Allodermanyssus sanguineus*) found on household rodents (mice, rats) and is very similar to, if not identical with, rickettsialpox described by American workers The causative agent has been named *Rickettsia dermacentroxenus murinus* (*D murinus*) (S M Kulagin; cited above) In the U S S R , the disease was first studied and differentiated clinically by I P. Drobinskii (1949-1950; cited above). In 1950 Zhdanov and co-workers and Kulagin (cited above) isolated the causative agent from the blood of human patients and described its experimental characteristics The studies of Vasil'eva and Golinevich demonstrated that the causative agent belonged to the tick-borne spotted fever group of rickettsiae Thus the rickettsiae showed a capacity for intranuclear multiplication when grown in chick embryo pulp on semisolid agar medium

By cross complement-fixation tests the antigenic structure of *D murinus* was found to be closely related to the rickettsiae of tick-borne typhus, *R conorii* (boutonneuse fever, Black Sea strains), and to the rickettsiae of North Asian tick-borne typhus (Altai and Krasnojarsk strains) but not related to the causative rickettsiae of epidemic typhus, murine typhus or Q fever Of particular in-

tive agent of North Asian tick-borne typhus Guinea pigs, mice, rats and chick embryos are susceptible to the rickettsial agent. A readily passaged, fatal, rickettsial pneumonia is produced in mice by intranasal inoculation of infected suspension.

The clinical picture in man is similar in all respects to that described for rickettsialpox by American workers and is essentially a benign, febrile disease characterized by the presence of primary lesions (*tâche noire*) at the site of mite bite and by the formation of a peculiar rash of papular-vesiculous type.

The disease has been reported to occur in certain areas of the southern zone of the European portion of the Soviet Union, principally in certain cities of the Southern Ukraine, but apparently not in Kharkov, since mention is made of the fact that house rats

acter and shows regular focal areas of infection in certain streets, blocks and houses with infection of persons based on contact with house rodents and their ectoparasites Drobinskii (cited above) has reported that cases of vesicular rickettsiosis may be observed throughout the year, but the data presented

strains from pools of infected mites, *Allodermanyssus sanguineus* (Hirst), which is believed to be the principal vector, and 1 strain from a wild house mouse (*Mus musculus*) trapped on the premises where cases of rickettsialpox had occurred. The human, mite and mouse strains were shown to be identical (Huebner et al., 1947). Complement-fixation tests on sera from recovered guinea pigs and from human patients have shown that a very close serologic relationship exists between the rickettsiae of rickettsialpox, Rocky Mountain spotted fever and tick-borne typhus However, these infections can be differentiated by using specific antigens prepared from washed suspensions of rickettsiae. Minor cross-reactions have been noted occasionally with murine typhus antigen (Rose, 1949) Guinea pigs recovered from rickettsialpox show partial although not complete protection against both spotted fever and tick-borne typhus Rickettsialpox is serologically related to other members of the spotted-fever group but not to epidemic typhus, scrub typhus, Q fever or Colorado tick fever. Consigli et al (1957) have reported that *R akari* (McCauley strain) possesses glutamic dehydrogenase, malic dehydrogenase, ATPase and ADPase systems and is able to synthesize citrate in the presence of oxalacetate. Furthermore, they have stated that *R akari* apparently has been able to multiply after 3 serial transfers on an artifical medium in vitro

Diagnosis

Rickettsialpox shows many similarities to tick-borne typhus. However, certain differences have been observed For instance, the rash in tick-borne typhus frequently involves the palms of the hands and the soles of the feet, which rickettsialpox seldom does. The rash in rickettsialpox becomes vesicular, whereas the rash of tick-borne typhus does so only rarely. Monkeys are susceptible to tick-borne typhus but apparently not to rickettsialpox Furthermore, sera from tick-borne typhus patients show a positive Weil-Felix reaction late in the course of the disease, whereas rickettsialpox patients apparently fail to produce agglutinins for Proteus strains of organisms Rickettsialpox may be differentiated from Rocky Mountain spotted fever not only on clinical grounds but also on serologic grounds, since sera from patients with either disease usually show a higher complement-fixation titer in the presence of washed homologous rickettsial antigens

Treatment

Rose (1952) has presented the results of antibiotic therapy in 26 patients with rickettsialpox among a total of 91 cases seen at the Columbia-Presbyterian Medical Center in New York City over a period of 4½ years In untreated cases of rickettsialpox the acute febrile stage usually lasted about 1 week, the fever and the associated symptoms gradually abating over the final 2 or 3 days of illness A single case (accidental laboratory infection) that was treated with streptomycin showed no therapeutic response, since complete defervescence and relief of symptoms did not take place until 8 days after clinical onset. In contrast, patients treated with chlortetracycline (9 cases), oxytetracycline (8 cases) or chloramphenicol (8 cases) uniformly showed a dramatic improvement within 48 hours, consisting primarily of relief of headache and malaise and a fall in temperature to normal levels The symptomatic improvement was accompanied by a rapid fading of the rash and disappearance of associated manifestations of the disease such as photophobia, cough, sore throat, nausea, vomiting, chills and sweats Additional experimental tests carried out in mice definitely showed chlortetracycline and oxytetracycline to be superior to chloramphenicol, particularly when antibiotic therapy was delayed until 24 to 48 hours after infection

Epidemiology

The disease rickettsialpox was first recognized in the Borough of Queens, New York City (Shankman, 1946), but it is now believed to have occurred and to have been listed among febrile conditions of unknown etiology for a number of years previously Additional cases of rickettsialpox have now been reported from a relatively circumscribed area within the United States, including New York City, Boston, Philadelphia, Cleveland and West Hartford (Conn) (cf. Hoeprich et al., 1956). The recovery of many strains of *Rickettsia akari* from the tissues of *Allodermanyssus sanguineus*, an ectoparasite of house mice (*Mus musculus*), has established the house mouse as the *reservoir* and the mite

rise in titer by the Weil-Felix test Nine of the 12 cases showed a titer of 1 320 or greater with Proteus OX19, and in 3 of these the titer was 1 160 or greater with OX2 No significant increase in titer was seen with Proteus OXK, which is usually observed in the case of scrub typhus

Mice and guinea pigs were found to be susceptible, with the latter showing fever and a scrotal reaction similar to that produced by murine typhus Guinea pigs sacrificed at the height of fever showed a slight enlargement of the spleen and the abdominal glands and an intense hemorrhagic congestion of the tunicae Rickettsiae were readily found in the cytoplasm but not the nuclei of large endothelial cells of the tunica vaginalis Rickettsial agglutination and complement-fixation tests carried out with the sera of 5 patients and antigens of epidemic typhus, murine typhus and tick-borne typhus (both boutonneuse and South African strains) indicated no serologic relationship Funder and Jackson (1946) and Plotz et al (1946) designated the disease North Queensland tick typhus

Both groups of workers showed that the rickettsiae could be readily cultivated in the yolk sac of fertile hens' eggs and in agar-slant tissue cultures, with the latter particularly giving rich growth and showing numerous intranuclear rickettsiae in infected cells thus indicating a possible relationship of this rickettsia to other members of the spotted-fever group It is worthy of note however, that even though the agar-slant tissue cultures on the average gave a richer growth of rickettsiae than the infected yolk sac, yet the Australian workers (Funder and Jackson, 1946) still found it more practicable to prepare their antigens by the yolk-sac method of rickettsial inoculation and cultivation

These findings are entirely in line with the author's own observations on the routine mass production of rickettsial vaccines and antigens in general Funder and Jackson (1946) showed that the 2 tick typhus strains (FIK and PHS) isolated by Andrew et al (1946) were serologically similar They further reported that the sera of convalescent patients fixed complement in the presence of antigens prepared from yolk sacs infected with the

workers in that they found guinea pigs re-

covered from the PHS tick typhus strain to

Thus, the serologic data obtained by the above workers indicated no relationship between the PHS strain of rickettsiae and those of the other diseases mentioned. However, subsequent studies carried out by Lackman and Parker (1948) with the same strain of Queensland tick typhus (PHS) definitely showed this strain to belong to the spotted-fever group of diseases Complement-fixation tests carried out with soluble antigens or rickettsial suspensions of Queensland tick typhus, and guinea pig sera prepared against various rickettsial strains showed relationship to, but not identity with, other members of the spotted-fever group, such as Rocky Mountain spotted fever, tick-borne typhus (boutonneuse and South African strains), rickettsialpox and maculatum disease. No relationship was shown between Queensland tick typhus, murine typhus and Q fever

On the basis of these studies, Philip in 1950 proposed the name *Rickettsia australis* for the causative agent of Queensland tick typhus (cited by Philip, 1953). Cross-immunity tests carried out in guinea pigs by Plotz et al (1946) showed some cross-resistance between Queensland tick typhus on the one hand and murine typhus and tick-borne typhus (South African strain) on the other However, the resistance induced against heterologous organisms was related to the period of time intervening between the initial infection and the subsequent challenge, and the question was raised whether this resistance to heterologous agents was evidence of specific immunity or of an acquired cellular resistance dependent upon other factors The importance of the time element in cross-immunity tests with rickettsial agents in guinea pigs was quite properly emphasized

Fenner (1946) carried out studies to determine what animals were natural hosts of infection and to isolate the rickettsiae from ticks At 2 sites on the Atherton tableland, North Queensland, 116 native animals were trapped and their ectoparasites and blood

cyclus, Ixodes tasmani and *Haemaphysalis humerosa.* Complement-fixation tests carried

show a very marked increase in morbidity during the spring-summer period (May to August) with a maximum in May and June According to Gear (1954), most of the cases resembling rickettsialpox in South Africa have contracted their infection in the bushveld, and thus it seems likely that the reservoir of infection will be found to be one of the wild rodents and not the house mouse, and the vector may be some mite other than *Allodermanyssus sanguineous* Thus far no strains of rickettsialpox have been isolated from patients or vectors in South Africa

Control Measures

No vaccine is available, although one probably could be made readily from infected chick-embryo yolk-sac tissues Preventive measures should include the eradication of all rodents known to be actual or potential hosts for the mite vector, as well as those measures necessary for killing the mites themselves

QUEENSLAND TICK TYPHUS

In 1946, Brody of Gordonvale, North Queensland, first described a case of febrile illness which, on the basis of clinical and serologic evidence appeared to belong to the category of tick-borne typhus The illness was associated with a widespread papular rash on the trunk and the extremities (the hands and the face were unaffected), no eschar was produced, but the rash persisted throughout the illness and was still faintly visible when the patient left the hospital on the 17th day This case was distinguished by a strongly positive Weil-Felix reaction with Proteus OX2 and a lesser reaction with Proteus OX19 (1 1,280 and 1:160, respectively, on the 21st day). No causative agent was isolated, but it was pointed out that the clinical course greatly resembled that generally described for "*fièvre boutonneuse* of the Mediterranean littoral"

In the same year (1946) Andrew et al. reported the isolation of 2 strains of rickettsiae (FIK and PHS strains) from 12 patients who apparently were infected by bites of ticks on the Atherton tableland in North Queensland, Australia This land is light savannah, interspersed with dense belts of rain forest, and carries a dense marsupial and rodent population that is heavily infested with ectoparasites—mites and ticks Four species known to bite man occur in this area· *Ixodes holocyclus, Haemaphysalis bancrofti, Boophilus annulatus* and *Rhipicephalus sanguineus.* Epidemiologic evidence thus far indicates that *Ixodes holocyclus* (both larval and adult forms) in all probability are primarily responsible for transmission of the disease to man Ten of the 12 cases occurred in August and September at the time of the early spring rains when *Ixodes holocyclus* is known to be most abundant on the tableland. In 2 cases adult ticks and in 1 case a larval *Ixodes holocyclus* were actually found attached to the patients at the time of hospital admission

The incubation period of the disease was estimated to be from 7 to 10 days. The first complaint was malaise and headache, which gradually increased in intensity, was bilateral, frontal and frequently retro-orbital An eschar, similar to that seen in scrub typhus, was seen in 9 cases Adenopathy was constantly present The regional glands draining the eschar were usually considerably enlarged, painful and tender and, in 11 patients, an examination of glands remote from these areas showed enlargement which persisted for 7 to 10 days The spleen was palpable in 2 cases Fever was continuous or remittent The temperature usually subsided by lysis after an average febrile period of 7 5 days (range 2 to 12 days).

A rash appeared in 11 cases In 9 of these, an average of 3 5 days elapsed from the time of onset of symptoms (range 1 to 6 days) No particular area of the body was noted as the starting point, although the trunk was always involved when the rash was first observed Great variation in the character of the rash occurred from case to case, and the individual lesions varied in color, size, degree of elevation and density of distribution In some cases they consisted of small, scattered, erythematous macules and papules In other cases the rash consisted of large pink papules similar to those commonly seen in scrub typhus In one of the latter cases the lesions became confluent on the trunk to form large purplish blotches of generalized rash also involving the face, the scalp and the palms of the hands. In general, though, the disease was mild, and none of the cases could be considered as severe All of the cases showed a

more closely related to tick-borne typhus than to spotted fever. Guinea pigs vaccinated against Rocky Mountain spotted fever are not protected against maculatum disease. No human cases of maculatum disease have been reported thus far.

TICK-BITE RICKETTSIOSES IN INDIA

In 1917, Megaw first suggested that forms of typhus other than louse-borne occurred in India when he described his own illness that occurred 3 weeks after a tick bite as "a case of fever, resembling Brill's disease." In 1921, he correlated this illness with a number of others reported from the same general area and by analogy with Rocky Mountain spotted fever suggested that the infections were possibly transmitted by ticks From these early reports, the expression "Indian tick-typhus" came into being

The disease was characterized by a diffuse macular erythematous rash that covered the entire body, including the palms of the hands and the soles of the feet The rash became brownish red and petechial within 2 days and faded by about the 12th day when the temperature returned to normal Following Megaw's

evidence that tick-borne typhus, related to the spotted fever group of organisms, occurs in India

More recently Kalra (1952) has reported the isolation of tick-typhus strains from ticks found infected in nature He reported the isolation of a strain from *Haemaphysalis leechi var. indica* at Imphal and from *Ixodes ricinus* at Almora, recovered as well were a number of strains from adult *Rhipicephalus sanguineus* collected in Srinagar, Kashmir (Rao, 1951) Apparently, most of these strains were lost. However, a number of *Rhipicephalus sanguineus* captured on the premises of a previous patient in Srinagar, Kashmir, northern India, were sent to Dr C B Philip of the Rocky Mountain Laboratory, Hamilton, Mont., through the courtesy of Maj. S L. Kalra and Capt. K. N A Rao of the Indian Army Typhus Research Detachment. The patient had been hospitalized the year before with a typhuslike infection following tick-bite

From 5 ticks that were injected into 2 guinea pigs, a rickettsial strain (Kashmir strain) was isolated which has been maintained by means of transfer with testicular washings and minced tunica tissue for more than 50

suggestive of spotted fever in the United States.

In 1943, Topping et al reported the results of complement-fixation tests on 3 sera obtained from patients in Mysore which indicated that the causative agent was more closely related to the spotted fever than to the typhus group Seaton and Stoker (1946) examined 40 sera from cases of the typhus group of fevers in India by the complement-fixation test, using Rocky Mountain spotted fever and murine typhus rickettsial antigens Eighteen of the sera fixed complement in the presence of the murine typhus rickettsial antigens with no demonstrable cross-fixation, while 16 sera fixed complement in the presence of the Rocky Mountain spotted fever antigen, with cross-fixation in 1 case only and that to a much lower titer Of the remaining 6 sera, 5 were negative with both rickettsial antigens, while the 6th was positive with both

guinea pigs by the bites of larvae, nymphs and adults of *R. sanguineus* infected in previous stages, although some of the test animals showed mild or inapparent infections as proved by challenge with virulent material Under similar donor conditions, under which infection with Rocky Mountain spotted fever would have been expected, *D andersoni* failed to become infected These results were comparable with those obtained in experimental tests of tick transmission of tick-borne typhus strains originating in the Mediterranean area as well as in South Africa The Kashmir strain was also grown in Zinsser flask tissue cultures from which smears showed rickettsiae in the nuclei of some infected cells, thus further proving its relationship to the spotted-fever group

According to Kalra (1952) tick typhus is present in many parts of India, but its inci-

out on the sera of animals with a Queensland tick typhus antigen (FIK strain) gave positive results with 8 out of 111 serum specimens Positive results were obtained from the following marsupials: *Isoodon obesulus* (short-nosed bandicoot), *Trichosurus vulpecula johnstonii* (Johnstone's oppossum),

Atherton uromys) Presumably, these animals had been infected at some time in the past with Queensland tick typhus rickettsiae. The majority of the infected animals were collected from a localized area of rain forest where 5 of the patients mentioned by Andrew et al. (1946) presumably contracted their infection.

In 1948, Streeten et al, reported 3 cases of tick typhus from South Queensland, the clinical histories of which were identical with the descriptions given by Andrew et al. (1946) No strains of rickettsiae were isolated, so that no comparison could be made with strains derived from North Queensland tick typhus. It was pointed out, however, that they are probably closely related, if not identical, for the same tick, *Ixodes holocyclus,* is notorious in both parts of Queensland for attacking man. More recently, Neilson (1955) has reported an additional case of tick typhus from South Queensland A strain of rickettsiae was isolated from the blood of Neilson's patient by inoculating weaned mice intraperitoneally (Pope, 1955) Mice were found to be more suitable than guinea pigs for primary isolation, and no difficulty was had in maintaining the strain (JC) apparently indefinitely in mice Serologic studies carried out with convalescent serum from the patient J. C as well as with sera from mice and guinea pigs recovered from infection with the JC strain of rickettsia showed that there was no relationship to the rickettsiae of murine typhus (*R. typhi*), scrub typhus (*R tsutsugamushi*) or Q fever (*Coxiella burnetii*). However, the convalescent guinea pig serum showed fixation of complement with *R. akari,* the causative agent of rickettsialpox and a

Queensland

Since the same disease apparently has been found to occur in South Queensland as well as in North Queensland, the author agrees with Neilson (1955) that a more appropriate name for the disease is Queensland tick typhus. Should the same disease later be proved to occur in other parts of Australia, then a still more appropriate name would be Australian tick typhus

MACULATUM DISEASE

Strains of a rickettsia mildly pathogenic for laboratory animals were isolated from specimens of the Gulf Coast tick, *Amblyomma maculatum,* collected from cattle near Cleveland, Texas, in 1937. The disease was produced in guinea pigs, and the cultural characteristics and immunologic relationships of the agent were first described by Parker et al (1939) The name "maculatum disease" was given to the syndrome produced in guinea pigs. Additional isolations were made from *A. maculatum* collected in Georgia (Parker, 1940), Mississippi and Texas (Lackman, et al, 1949).

The rickettsiae grow readily in the yolk sac of the developing chick embryo The guinea pig is the laboratory animal of choice; intraperitoneal injection of testicular washings is used in making transfers The disease in the guinea pig is characterized by a mild, never fatal, infection, a short febrile period and a swollen, pinkish scrotum—not infrequently there is only fever or only a scrotal reaction without fever. Monkeys, rabbits and white rats are mildly susceptible, while the Sawatch meadow mouse is quite susceptible (Parker, 1940). There is good reciprocal cross-immunity in guinea pigs between maculatum infection, Rocky Mountain spotted fever (both Montana and Brazilian strains) and tick-borne typhus. Guinea pigs recovered from murine or louse-borne typhus are usually resistant to maculatum infection, but reverse cross-immunity does not take place, nor is there any relationship between maculatum disease and Q fever Complement-fixation studies carried out by Lackman, Parker and Gerloff (1949) confirmed the fact that maculatum disease rickettsiae belong to the spotted fever group but are antigenically different from the rickettsiae of Queensland tick typhus, tick-borne typhus (South African strain), Rocky Mountain spotted fever and rickettsialpox On the basis of the clinical picture induced in the guinea pig, Parker (1940) stated that the infection is apparently

tonitis accompanied by multiplication of rickettsiae in the mesothelial cells

In man, the incubation period is 3 to 6 days, with possible variations within the range of 2 to 7 days In many cases, the prodromal symptoms are a general indisposition, congestion, headache, muscular pains, and loss of appetite, while in other cases the disease begins without a prodromal period, with a considerable elevation in temperature Fever develops rapidly and reaches a maximum on the 3rd or 4th day of the disease, with a temperature elevation to 40° C or higher. The mean duration of fever is 8 to 10 days, usually terminating in lysis lasting 2 to 3 days The fever curve is most often of the remissive type, but occasionally the fever may be constant.

The primary lesion (*tâche noire*) is regularly observed and takes the form of a small, dense infiltrate, covered with a brown crust and surrounded by a reddish areola Histologically, it is characterized by the presence of a central wedge-shaped necrosis The primary lesion is usually accompanied by the development of regional lymphadenitis, sometimes with enlargement of the lymph nodes to the size of a pigeon's egg The localization of the primary lesion varies for example, when the patient is bitten by *D nuttalli*, the bites appear more often on the hairy part of the head, on the neck and on the shoulder area, whereas after the bite of *H concinna* the lesion is found most often on the abdomen The localization of the *tâche noire* is also determined to a considerable extent by the character of the clothing and the footwear, e g , by the degree of protection against tick-bites

The rash appears as a constant symptom of the disease, it appears sometimes by the 2nd day but usually on the 4th to the 5th days of the disease The eruption is of a polymorphic, roseoloid-papular character, with hemorrhagic transformation In many cases a copious rash covers the whole body and the extremities, in other cases the rash is less abundant, mostly localized on the chest, the back and the inner surfaces of the extremities The rash may also develop on the face, the palms and the soles The rash persists after the fever has dropped, and the pigmentation remains for a long time

TABLE 39 HOSTS OF 5 NORTH ASIAN TICKS

D nuttalli
Adults cows, sheep, horses, man
Nymphs and larvae vole, Siberian marmot (suslik), hamster, rats

D silvarum
Adults large animals both domestic and wild
Nymphs and larvae voles, mice, rats, chipmunks, hare, badger, Siberian weasel, woodcock, black bird, sparrow, greenfinch and blue magpie

D marginatus and
D pictus
Adults cattle, horses, swine, dogs and man
Nymphs and larvae narrow skulled vole, mice, hamsters, white weasel, striped squirrel, chipmunk, hawk

H concinna
Adults large animals both domestic and wild, man
Nymphs and larvae voles, mice, striped squirrels, hedgehog, greenfinch, black bird, wood cock, quail, sparrow, and man (for nymphs)

Central nervous system symptoms are generally limited to headaches, persistent and often agonizing The patients also often suffer from muscular pains as well as pains in the lumbar region However, a typhoid state and delirium are seldom observed and are not characteristic of this disease The spleen is not regularly palpable, but it may become so in the later course of a more serious illness The eyes reveal conjunctivitis and injection of the sclerae The general character of the disease is benign, with a completely favorable prognosis Relapses are not observed A positive Weil-Felix reaction to Proteus OX19 antigen appears late in the course of the disease

Specific complement-fixing antigens have been prepared from chick embryo cultures of rickettsiae Six species of ticks have been implicated in the transmission of the disease, these being *Dermacentor nuttali, D silvarum, D marginatus, D pictus, Haemaphysalis concinna*, and *H punctata*. Hosts of these ticks are given in Table 39 Their natural habitats are open areas of steppe, plains, or meadow land (*D nuttalli, D marginatus, D pictus*), hilly regions with occasional wooded shrubbery (*D silvarum*), or well-illuminated, slightly hilly, marsh land and damp places

dence is the lowest of all the typhus group of fevers. The recorded incidence of tick typhus in the literature is so meager that no statement can be made about its seasonal prevalence Surveys of the Seoni and the Chindwara forests in the Central Province showed that tick density increased during the rains, remained high during the mild winter but dropped rapidly with the approach of the hot, dry summer. It is probable that, at least in these parts, the seasonal incidence of tick-typhus follows the same course For additional comments on rickettsial diseases in India see Soman (1954).

A summation of all evidence shows that the Kashmir strain of "Indian tick-typhus" is a member of the spotted-fever group and indicates that it has a much closer relationship to the tick-borne typhus of the Mediterranean area (boutonneuse fever, causative agent, *R conorii*) than to Rocky Mountain spotted fever (causative agent, *R. rickettsii*) Additional studies are needed to determine the exact relationship of the Kashmir and other tick-typhus strains isolated in India to the other recognized rickettsial agents of the spotted-fever group.

TICK-BORNE RICKETTSIOSES *IN NORTHERN ASIA*

The available knowledge concerning rickettsial infections transmitted by ticks in northern Asia is well summarized by Zdrodovskii and Golinevich (1956a) These diseases are produced by rickettsiae of the species *Dermacentroxenus sibericus* (*Rickettsia sibiricus*) and belong to the tick-borne spotted-fever group *The clinical picture is characterized by a relatively mild, acutely febrile* course, the presence of a primary lesion (*tâche noire*), and a maculopapular rash. Various species of ixodid ticks of the genera *Dermacentor* and *Haemaphysalis*, together with certain small wild animals, constitute the reservoir of this disease. The first clinical observations were made in 1934-1936 by the Soviet investigators Mill, Antonov and Neishtadt, and Fedukovich. It was found that the disease first described in the Krasnojarsk region was the same as that found in Western, Central and Eastern Siberia and in the territories of the Far East. In addition it has been found in some localities of Central Asia and in the Mongolian Peoples Republic.

Cross-immunity experiments showed that guinea pigs that had been experimentally infected with *R. sibiricus* were fully resistant to subsequent infection with tick-borne typhus rickettsiae (boutonneuse fever, *R conorii*), isolated from dog ticks (*Rhipicephalus sanguineus*) on the coast of the Black Sea Serologic analysis of the antigenic structure showed that *R. sibiricus* is closely related to *R. conorii* and is different from the causative agents of epidemic typhus, murine typhus and Q fever. Two strains of *R. sibiricus* (Altai and Krasnojarsk strains) were shown to *multiply intranuclearly in infected epithelial* cells of the testicular membranes of guinea pigs showing periorchitis, demonstrating that these rickettsiae belong to the tick-borne spotted fever group The rickettsiae give scanty *growth in the yolk sac of the developing* chick embryo (this is the typical picture obtained with all rickettsiae of the spotted-fever group) but grow luxuriantly in minced chick embryo-agar substrate tissue cultures Rhesus monkeys infected subcutaneously or intraperitoneally show an incubation period of 3 to 4 days and fever lasting 5 to 7 days The infection is accompanied by a positive Weil-Felix reaction with Proteus OX19 antigen Male guinea pigs show considerable susceptibility, responding to infection with fever and an accompanying scrotal reaction Rabbits inoculated intraperitoneally show practically no signs of illness, but when injected intratesticularly respond with a pronounced specific orchitis. Rickettsial suspensions inoculated into the anterior chamber of the eye (rabbit) give rise to specific iridocyclitis of long duration. Intradermal inoculation of rabbits with rickettsial suspensions gives rise to *the development of a local inflammatory reaction* with subsequent necrosis. This reaction begins, on the average, after 48 hours, reaches its maximum and subsides within 9 to 10 days, with the formation of a scab Recovered rabbits show a positive Weil-Felix reaction with Proteus OX19. Rats inoculated intraperitoneally show a febrile reaction of short duration, with rickettsial peritonitis (as in boutonneuse fever), but without a prolonged maintenance of rickettsiae in the brain. Mice infected intranasally show a specific rickettsial pneumonia Intraperitoneal infection of mice produces a slight or moderately pronounced form of rickettsial peri-

causative agent has been named *Rickettsia pavlovskyi* in honor of the famed Soviet academician, E. N. Pavlovski. The morphologic features of *R. pavlovskyi* are characterized by high pleomorphism and both intracytoplasmic and intranuclear tendencies. It is claimed that the rickettsiae may be readily filtered through Berkefeld filters (porosity not stated), and that the filtrates retain their activity for more than a year at 30° C. Convalescent human sera possess the capacity to neutralize the cutaneous reaction in rabbits induced by an intracutaneous injection of infected mouse lung, rickettsial suspensions. The disease appears to be very widely spread in nature.

In the first endemic area, rickettsial strains were isolated from the blood and the organs of gophers, common weasels, field and forest mice. Rickettsial strains also were isolated from 6 groups of Ixodid ticks, *Ixodes laguri laguri*, 7 groups of larvae of the "red-bodied tick" *Trombicula autumnalis Shaw* and *Neoschoengastia*, 1 group of the "nymph red-bodied ticks" *Trombicula autumnalis* with a predominance of Neoschoengastia, 2 groups of "gamasid ticks" *Liponyssus arvicolae Zem* and *Haemogamasidae* from the nests of weasels and starlings, and 6 groups of fleas, mainly *Ctenophthalmus orientalis*. In the second endemic area, rickettsial strains were isolated from the blood and the tissues of both red and common mice (vole). Rickettsial strains were stated to be isolated also from 18 groups of "gamasid ticks" *Laelaps cleth-*

met months), 2 groups of "red-bodied ticks" *Trombicula zachvatkini*, and 3 groups of fleas, mostly *Ceratophyllus turbidus* and *Ctenophthalmus agyrtes*. Rickettsial strains were also isolated from 4 groups of *Dermacentor pictus* that were allowed to feed on guinea pigs. In the third endemic area, rickettsial strains were isolated from the blood and the organs of yellow-breasted mice, red and common voles. Rickettsial strains were also isolated from 10 groups of "gamasid ticks" containing *Laelaps agilis* (Koch), *Liponyssus talpae Zem*, and *Liponyssus arvicolae Zem*, a single group of "red-bodied ticks" *Trombicula autumnalis*, 3 groups of ixodid ticks, *Ixodes ricinus*, obtained from cows, 3 groups of fleas, of which the predominant species were *Ctenophthalmus assimilis*, *Ctenophthalmus agyrtes* and *Ctenophthalmus solutus*. In addition, it was demonstrated that rickettsiae could be transmitted from generation to generation in the ticks *Dermacentor pictus* and *Ixodes ricinus*.

MISCELLANEOUS

"Parumapertus Strains" of Spotted Fever

In 1953, Philip and Hughes reported the isolation of rickettsial strains related to the spotted fever group from lots of the "rabbit Dermacentor" tick, *D. parumapertus* collected in northern Nevada. Further studies reported more recently by Philip et al. (1955) indicate that the "*D. parumapertus* strains" apparently show a "third divergence in virulence" of the rickettsial agents recovered from North American ticks other than from the classic vectors, *D. andersoni* and *D. variabilis*. Rickettsiae representing the other two lines of divergence in virulence have been isolated from *Amblyomma maculatum*, and *Haemaphysalis leporis-palustris*, the tick vectors of which, [redacted] a rule, infest hosts other than man. Further studies will be needed to elucidate the exact relationships of these strains of rickettsial agents to one another.

REFERENCES

(For complete references, see also 2nd edition, Chapter 36.)

Aikawa, J. K., and Harrell, [redacted] T., 1953, Effect of cortisone acetate on experimental Rocky Mountain spotted fever in the guinea pig, Proc. Soc. Exper. Biol. & Med. *82*, 698-701.

Alexander, R. A., and Mason, J. H., 1939, Studies of the rickettsias of the Typhus-Rocky-Mountain-spotted-fever group in South Africa. II. Morphology and cultivation, Onderstepoort J. Vet. Sc. [redacted] Anim. Indust. *13*, 25-39.

Allen, A. C., and Spitz, S., 1945, A comparative study of the pathology of scrub typhus (tsutsugamushi disease) and other rickettsial diseases, Am. J. Path. *21*, 603-681.

Amaral, A. do, and Monteiro, J. L., 1932, Ensaio de classificacao das rickettsioses a luz dos nossos actuaes conhecimentos, Mem. Inst. Butantan *7*, 343-376.

Andrew, R., Bonnin, J. M., and Williams, S., 1946, Tick typhus in North Queensland, M. J. Australia *2*, 253-258.

Anigstein, L., and Bader, M. N., 1943, Investigations on rickettsial diseases in Texas. Part I. Epidemiological role of ticks common to the Gulf Coast in

(*H. concinna*). The period of greatest activity of the ticks on large animals occurs during the spring, but to a certain extent the attacks of ticks may be observed also during the following summer months It is also important to note that the adult ticks show the greatest incidence of attacking man during the spring and early summer months.

INFECTIOUS NEPHROSONEPHRITIS OF THE USSR

Recently, Korshunova (1954) has reported a disease in man, apparently rickettsial in nature, called "infectious nephrosonephritis," "kidney rickettsiosis" or "rickettsiosis with inflammation of the kidneys" Starting about 1950, the disease has been reported in 3 endemic areas of the Soviet Union, separated by great distances and covering zones of diverse topography The first area is in the southwest USSR, in the level steppes (plains), broken by low troughs of dried-up rivers and dry gullies The second area is in the central part of the Union, in a zone of mixed forest predominantly oak, with a high grassy cover, interlaced with deep ravines The third area is on the slopes of the Trans-Carpathians, in a zone of broad-leaved forest predominantly beech and oak In all 3 endemic regions the disease appears about the middle of June and continues to the middle of August with the peak in July.

The clinical illness is briefly characterized as follows a rapid onset, temperatures elevated to 38.5° to 40 0° C., flushed face and cheeks, sore throat, injection of the conjunctivae and the vessels of the sclerae, and in some cases violent vomiting. As a rule, the patients complain of pains in the back, sometimes in the abdomen. There is general malaise and great weakness with slow recovery. The urine shows albumin, erythrocytes, leukocytes and casts. The blood picture shows leukopenia in the early phases and leukocytosis in the later stages On pathologic-anatomic examination there is primarily an involvement of the kidneys, so characteristic, that by this symptom alone it is possible to diagnose the disease correctly. The configuration of the kidneys is changed, especially the right one; they are enlarged, sometimes to such an extent that the capsule is ruptured; the pyramids are dark red in color in contrast with the background of the paler cortical layer In all cases there is also noted an involvement of the anterior portion of the pituitary.

The disease has been established in guinea pigs by intraperitoneal inoculation and in mice by intracerebral and intranasal infection. Guinea pigs show an incubation period of 3 to 6 days, followed by a febrile period of 4 to 6 days with a rise in temperature to 40° C. or higher; after this, the temperature subsides rapidly or moderately. Most of the animals show a second temperature rise on the 14th to 18th days.

The pathologic picture in the guinea pig is characterized by congested lungs, liver, testes and vaginal walls. There is enlargement and inflammation of the suprarenals with necrotic centers. Frequently, the liver shows a yellowish tinge. The kidneys show "uneven inflammation" Preparations of homogenates from scrotal membranes show intracellular and extracellular rickettsiae, distinguished by marked polymorphism The disease may be maintained in guinea pigs by passage of brain or other tissues. Mice injected intracerebrally show symptoms beginning on the 8th to 10th days, marked by inactivity, shortened gait, pilo-erection and sometimes paralysis of the hind limbs The disease, whether caused by intracerebral injection or by intranasal inoculations, is always fatal in mice Mice inoculated intranasally with suspensions of infected guinea pig tissues develop a rickettsial pneumonia on the 6th to the 10th days On subsequent mouse passage, the incubation period shortens to 3 to 4 days. Homogenates of lung tissue, stained by the Romanovsky method, show great quantities of rickettsiae Rabbits inoculated intradermally, with rickettsial suspensions of infected mouse lung tissue, show a skin reaction with necrosis Rabbits injected intratesticularly develop an orchitis lasting 3 to 5 days. White rats fail to show any signs of the disease.

From the first endemic area, 8 isolates of rickettsiae were made from the blood of 20 patients, and 3 strains were isolated from the brains of 8 fatal cases In the second endemic area, 5 rickettsial strains were isolated from the blood of 10 cases studied In the third endemic area, 8 isolates of rickettsiae were made from the blood of 20 cases; 3 isolations were made from the liver, the pituitary and the cerebellum of a single fatal case. The

causative agent has been named *Rickettsia pavlovskyi* in honor of the famed Soviet academician, E N. Pavlovski. The morphologic features of *R pavlovskyi* are characterized by high pleomorphism and both intracytoplasmic and intranuclear tendencies It is claimed that the rickettsiae may be readily filtered through Berkefeld filters (porosity not stated), and that the filtrates retain their activity for more than a year at 30° C Convalescent human sera possess the capacity to neutralize the cutaneous reaction in rabbits induced by an intracutaneous injection of infected mouse lung, rickettsial suspensions The disease appears to be very widely spread in nature

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REFERENCES

(For complete references, see also 2nd edition, Chapter 36)

Aikawa, J K., and Harrell, G T, 1953, Effect of cortisone acetate on experimental Rocky Mountain spotted fever in the guinea pig, Proc Soc Exper Biol & Med *82*, 698-701

Alexander, [illegible] A, and Mason, J H, 1939, Studies of the rickettsias of the Typhus-Rocky-Mountain-spotted-fever group in South Africa II Morphology and cultivation, Onderstepoort J Vet Sc & Anim Indust *13*, 25 39

Allen, A C, and Spitz, S, 1945, A comparative study of the pathology of scrub typhus (tsutsugamushi disease) and other rickettsial diseases, Am J Path *21*, 603-681

Amaral, A do, and Monteiro, J L, 1932, Ensaio de classificacao das rickettsioses a luz dos nossos actuaes conhecimentos, Mem Inst Butantan *7*, 343-376

Andrew, R, Bonnin, J M, and Williams, S, 1946, Tick typhus in North Queensland, M J Australia *2*, 253-258

Anigstein, L, and Bader, M N, 1943, Investigations on rickettsial diseases in Texas Part I Epidemiological role of ticks common [illegible] the Gulf Coast in

relation to local spotted fever, Texas Rep Biol. & Med *1*, 105-116

Anigstein, L, and Bader, M N, 1945, Para-aminobenzoic acid—its effectiveness in spotted fever in guinea pigs, Science *101*, 591-592

Anigstein, L, Whitney, D M, and Beninson, J, 1948, Aureomycin—a new antibiotic with antirickettsial properties its effect on experimental spotted fever and epidemic typhus, Ann New York Acad Sc *51*, 306-317

Aquilina, J T, Rosenberg, F, and Wuertz, R L, 1952, Nodal tachycardia in a case of Rocky Mountain spotted fever, Am Heart J *43*, 755-760

Arney, W C, 1952, Cortisone and aureomycin in the treatment of a case of Rocky Mountain spotted fever, North Carolina M J *13*, 20-22

Badger, L F, 1933, Rocky Mountain spotted fever. susceptibility of the dog and sheep to the virus, Pub Health Rep *48*, 791-795

Baker, G E, 1951, Rocky Mountain spotted fever, M Clin North America, *35*, 907-930

Barker, L P, 1949, Rickettsialpox—clinical and laboratory study of twelve hospitalized cases, J A M A *141*, 1119-1123

Bell, E J, and Pickens, E G, 1953, A toxic substance associated with the rickettsias of the spotted fever group, J Immunol *70*, 461-472

Bengtson, I A, 1948 Family rickettsiaceae *in* Breed, R S, Murray, E G D, and Hitchens, A P (eds), Bergey's Manual of Determinative Bacteriology, ed 6, pp 1083-1099, Baltimore, Williams & Wilkins

Berlin, L, and Thomas, M H, 1948, Neurologic sequelae of Rocky Mountain spotted fever, Univ Michigan Hosp Bull *14*, 22-23

Blanc, G, and Caminopetros, J, 1932, Études epidemiologiques et expérimentales sur la fièvre boutonneuse, faites a l'Institut Pasteur d'Athènes, Arch Inst Pasteur de Tunis *20*, 343-394

Brody, J, 1946, A case of tick typhus in north Queensland, M J Australia *1*, 511-512

Brumpt, E, 1927, Precis de parasitologie, ed 4, 1452 pp, Paris, Masson (See p 872)

———, 1932, Longevité du virus de la fievre boutonneuse (*Rickettsia conori*, n sp) chez la tique, *Rhipicephalus sanguineus*, Compt Rend Soc de Biol *110*, 1199-1202

Bustamente, M E, and Varela, G, 1943, Una nueva rickettsiosis en Mexico, Rev Inst Salub y Enferm. Trop *4*, 189-210

Bustamente, M E, Varela, G, and Ortiz Mariotte, C, 1946, II Estudios de fiebre manchada en Mexico Fiebre manchada en La Laguna, Rev Inst Salub y Enferm Trop *7*, 39-48

Calero, M C, Nunez, J M, and Silva-Goytia, R, 1952, Rocky Mountain spotted fever in Panama report of two cases, Am J. Trop Med *1*, 631-636

Cawley, E P, and Wheeler, C E, 1957, Rocky Mountain spotted fever, J A M A *163*, 1003-1007

Chang, R. S, Murray, [redacted] S, and Snyder, J C, 1954, Erythrocyte-sensitizing substances from rickettsiae of the Rocky Mountain spotted fever group, J Immunol *73*, 8-15

Charters, A D, 1946, Tick-typhus in Abyssinia, Tr Roy Soc Trop Med & Hyg *39*, 335-342

Consigli, R A, Paretsky, D, and Downs, C M, 1957, Studies on the physiology of *Rickettsia akari* II Some observations on attempts to cultivate *R akari* in vitro, Bact. Proc *1957*, p. 67.

Cooke, C., 1948, Rocky Mountain spotted fever treated with Aureomycin, J A M A *138*, 885.

Cooley, [redacted] A, 1938 The genera *Dermacentor* and *Otocentor* (Ixodidae) in the United States, with studies in variation, Nat Inst Health Bull *171*, 89

Cooley, R A, and Kohls, G M, 1944, The genus *Amblyomma* (Ixodidae) in the United States, J Parasitol *30*, 77-111

Cox, H R, 1938, Use of yolk sac of developing chick embryo as medium for growing rickettsiae of Rocky Mountain spotted fever and typhus groups, Pub Health Rep *53*, 2241-2247

———, 1941, Cultivation of rickettsiae of the Rocky Mountain spotted fever, typhus, and Q fever groups in the embryonic tissues of developing chicks, Science *94*, 399-403

———, 1948 The preparation and standardization of rickettsial vaccines *in* Moulton, F R (ed) Rickettsial Diseases of Man, pp 203-214, Washington, D C, Am Assoc Advancement Sc

Dauer, C C, 1957, Personal communication, National office of vital statistics, U S Pub Health Service, Washington, D C.

Davis, G E, 1942, The Rocky Mountain spotted fever rickettsia in the tick genus *Ornithodoros*, Proc 6th Pacific Sc Congr *5*, 577-579

———, 1943a, The tick *Ornithodoros rudis* [redacted] a host to the rickettsiae of the spotted fevers of Colombia, Brazil and the United States, Pub Health Rep *58*, 1016-1020

———, 1943b, Experimental transmission of the rickettsiae of the spotted fevers of Brazil, Colombia, and the United States by the argasid tick *Ornithodoros nicollei*, Pub Health Rep *58*, 1742-1744

Davis, G E, and Parker, R R, 1934, Comparative experiments on spotted fever and boutonneuse fever (I), Pub Health Rep *49*, 423-428

Davis, G E, Wynns, [redacted] L, and Beck, M D, 1941, Relapsing fever *Ornithodoros parkeri* a vector in California, Pub Health Rep *56*, 2426-2428

De Rodaniche, [redacted] C, and Rodaniche, A, 1950, Spotted fever in Panama, isolation of the etiologic agent from a fatal case, Am J Trop Med *30*, 511-517

Dezest, G, 1953, Les fievres boutonneuses du Cameroun sont graves, Bull Soc Path Exot *46*, 353-359

Dias, E, and Martins, A V, 1939, Spotted fever in Brazil a summary, Am J Trop Med *19*, 103-108

Dolgopol, V B, 1948, Histologic changes in rickettsialpox, Am J Path *24*, 119-133

Durand, P, 1932a, La réaction de Weil-Felix dans la fièvre boutonneuse, Arch Inst Pasteur de Tunis, *20*, 395-421

———, 1932b, Rôle du chien comme réservoire de virus dans la fièvre boutonneuse, Arch Inst Pasteur de Tunis *21*, 239-250

Durand, P, and Laigret, J, 1932, Sensibilité de certaines rongeurs au virus de la fievre boutonneuse, Arch Inst Pasteur de Tunis 20, 426-429

Dyer, R E, Badger, L F, and Rumreich, A, 1931, Rocky Mountain spotted fever (eastern type) Transmission by the American dog tick (*Dermacentor variabilis*), Pub Health Rep 46, 1403-1413

Ewing, H E, 1942, A second introduced rat mite becomes annoying to man, Proc Helminthol Soc, Washington 9, 74-75

Fenner, F, 1946, The epidemiology of North Queensland tick typhus natural mammalian hosts, M J Australia 2, 666 668

Finland, M, Collins, H S, Gocke, T M, and Wells, E B, 1949, Present status of Aureomycin therapy, Ann Int Med 31, 39-52

Fuller, H S, 1954, Studies of rickettsialpox III Life cycle of the mite vector, *Allodermanyssus sanguineus* Am J Hyg 59, 236-239

Funder, J F, and Jackson, A V, 1946, North Queensland tick typhus a comparative study of the rickettsia with that of murine typhus, M J Australia 2, 258-263

Gear, J, 1954, The rickettsial diseases of southern Africa A review of recent studies, South African J Clin Sc 5, 158-175

Gear, J, and Harington, A L, 1949, Tick bite fever Two cases treated with Aureomycin (Lederle), a new antibiotic, South African M J 23, 507-508

Gear, J H S, 1950, Rickettsioses dans l'Union Sud Africaine OMS, doc WHO/typhus/5

Gibbons, R J, 1942, Rocky Mountain spotted fever in Canada, Proc 6th Pacific Sc Congr 5, 573-575

Glasgow, R D, and Collins, D L, 1948, Ecological, economic and mechanical considerations relating to the control of ticks and Rocky Mountain spotted fever on Long Island, J Econ Entomol 41, 427-431

Gould, D J, and Miesse, M L, 1954, Recovery of a rickettsia of the spotted fever group from *Microtus pennsylvanicus* from Virginia, Proc Soc Exper Biol & Med 85, 558-561

Greenberg, M, Pellitteri, O, Klein, I F, and Huebner, R J, 1947, Rickettsialpox—a newly recognized rickettsial disease II Clinical observations, J A M A 133, 901-906

Hamilton, H L, 1945, Effect of p-aminobenzoic acid on growth of rickettsiae and elementary bodies, with observations on mode of action, Proc Soc Exper Biol & Med 59, 220-226

Harrell, G T, 1949, Rocky Mountain spotted fever, Medicine 28, 333-370

———, 1952, Treatment of Rocky Mountain spotted fever with antibiotics, Ann New York Acad Sc 55, 1027-1042

Harris, B P, 1952, Aureomycin in tick typhus East African M J 29, 403 410

———, 1953, Aureomycin in tick typhus second series, East African M J 30, 99-110

Hass, G M, and Pinkerton, H, 1936, Spotted fever II An experimental study of fièvre boutonneuse, J Exper Med 64, 601-623

Havens, F Z, Jr, and Stickney, J M, 1950, Rocky Mountain spotted fever successfully treated with Aureomycin report of a case, Proc Staff Meet Mayo Clin 25, 338-342

Hearle, E, 1938, The ticks of British Columbia, Sc Agric (Ottawa) 18, 341-354

Heilig, R, and Naidu, V R, 1941, Endemic typhus in Mysore, Indian M Gaz 76, 705-710

———, 1942, Further experiences on endemic typhus in Mysore, Indian M Gaz 77, 338-342

Herms, W B, and Howell, D C, 1936, The western dog tick, *Dermacentor occidentalis* Neum, a vector of bovine anaplasmosis in California, J Parasitol 22, 283-288

Hildick-Smith, G, 1949, Tick bite fever. report of a case treated with Aureomycin, South African M J 23, 702-703

Hoeprich, P D, Kent, G T, and Dingle, J H, 1956, Rickettsialpox report of a serologically proved case in Cleveland, New England J Med 254, 25-27

Huebner, R J, Jellison, W L, and Armstrong, C, 1947, Rickettsialpox—a newly recognized rickettsial disease V Recovery of *Rickettsia akari* from a house mouse (*Mus musculus*), Pub Health Rep 62, 777-780

Huebner, R J, Jellison, W L, and Pomerantz, C, 1946, Rickettsialpox—a newly recognized rickettsial disease IV Isolation of a rickettsia apparently identical with the causative agent of rickettsialpox from *Allodermanyssus sanguineus*, a rodent mite, Pub Health Rep 61, 1677-1682

Huebner, R J, Stamps, P, and Armstrong, C, 1946, Rickettsialpox—a newly recognized rickettsial disease I Isolation of the etiological agent, Pub Health Rep 61, 1605-1614

Jadin, J, 1951 Les rickettsioses du Congo Belge et du Ruanda-Urundi Thesis for appointment in higher education, Catholic University of Louvaine Editions Nauwelaerts, 2 Place Cardinal Mercier, Louvaine, Belgium, 108 pp

Jellison, W L, 1945, The geographical distribution of Rocky Mountain spotted fever and Nuttall's cottontail in the western United States, Pub Health Rep 60, 958-961

Joint OIHP/WHO Study Group on African Rickettsioses Report on the first session World Health [illegible]

[illegible] 6, [illegible]

Kiselev, R I, and Volchanetskaia, 1954 The significance of the tick *Allodermanyssus sanguineus* [illegible]

[illegible] Inst Epidemiol & Microbiol Leningrad, State Publishing House of Medical Literature, Medgiz pp 248-252 (Translations courtesy of Dr B Szumski and Mr F Danga, American Cyanamid Co, Pearl River N Y)

Kohls, G M, 1948 Vectors of rickettsial diseases in Moulton, F R, (ed), Rickettsial Diseases of Man, pp 83-96, Washington, D C, Am Assoc Advancement Sc

Korshunova, O S, 1954 Experimental data on the study of infectious nephroso-nephritis *in Natural* Nidi of Human Diseases and Regional Epidemiology, Proc Joint Conference, USSR Ministry of Public Health, the USSR Acad Med Sc, Gamalea *Inst Epidemiol & Microbiol Leningrad, State* Publishing House of Medical Literature, Medgiz pp. 239-243 (Translations courtesy of Dr S Szumski and Mr F Danga, American Cyanamid Company, Pearl River, N Y)

LaBoccetta, A C, Israel, H L, Perri, A M, and Sigel, M M, 1952, Rickettsialpox Report of four apparent cases in Pennsylvania *Am J. Med 13,* 413-422.

Lackman, D, and Parker, R R, 1948, The serological characterization of North Queensland tick typhus, *Pub Health Rep. 63, 1624-1628*

Lackman, D B, and Gerloff, R. K, 1953, The effect of antibiotic therapy upon diagnostic, serologic tests for Rocky Mountain spotted fever, Pub Health Lab *11,* 97-99

Lackman, D B, Parker, R R, and Gerloff, R K, 1949, Serological characteristics of a pathogenic rickettsia occurring in *Amblyomma maculatum,* Pub Health Rep *64,* 1342-1349

Le Gac, P, *1953,* Research on rickettsialpox in Oubangui-Chari, West African M J *2,* 42-50

Le Gac, P, Giroud, P, *Le Henaff, A*, and Baup, G, 1952, Epidémie familiale de rickettsiose varicelliforme dans un village de l'Oubangui-Chari (A E F), Bull Soc Path Exot *45,* 19-23

Lillie, R D, 1941, Pathology of Rocky Mountain spotted fever I The pathology of Rocky Mountain spotted fever II The pathologic histology of Rocky Mountain spotted fever in the rhesus monkey *Macaca Mulatta* Nat Inst Health Bull *177,* 59

McCroan, J E, Ramsey, R L, Murphy, W J, and *Dick, L S, 1955, The status of Rocky Mountain* spotted fever in the southeastern United States, Pub Health Rep *70,* 319-325

de Magalhães, O, and Rocha, A, 1942, Tifo exantimatico do Brasil, papel do cão (*C familiaris*) na constituição dos focos da moléstia, Brasil-Med *56,* 370-377

*Maxey, E E, 1899, Some observations on the so-*called spotted fever of Idaho, Med Sentinel, Portland, Oregon *7,* 433-438

Megaw, J W D, 1917, A case of fever resembling Brill's disease, Indian M Gaz *52,* 15-18

———, 1921, A typhus-like fever in India, possibly transmitted by ticks, Indian M Gaz *56,* 361-371

Miller, J K, 1950, Rocky Mountain spotted fever on Long Island, Ann Int Med. *33,* 1398-1406

Monteiro, J L, 1931, I Comportamento experimental e propriedades do virus 2 A Rickettsia brasiliensis e suas relacoes com a infeccao Mem *Inst. Butantan 6,* 3-135

Neilson, G H, 1955, A case of Queensland tick typhus, M J Australia *42*(1) 763-764

Olmer, D, and Olmer, J, 1956, Epidemie des rickettsioses sur le littoral Méditerranéen, la fièvre boutonneuse, Rev Path Gen, Paris *56,* 80-92

Parker, R R, 1923, Maintenance of the virus of Rocky Mountain spotted fever in nature with particular reference to conditions in the Bitter Root Valley, *Montana State* Board of Health Special Bull *26,* 33-40

———, 1938, Rocky Mountain spotted fever, J.A.M.A *110,* 1185-1188, 1273-1278.

———, 1940, A pathogenic rickettsia from the gulf coast tick, *Amblyomma maculatum,* Proc. Third Internat. Congr. Microbiol pp 390-391.

Parker, R R, Kohls, G M, Cox, G W., and Davis, G E, 1939, Observations on an infectious agent from *Amblyomma maculatum,* Pub Health Rep *54,* 1482-1484

Parker, R. R, Kohls, G M, and Steinhaus, E A, 1943, Rocky Mountain spotted fever spontaneous infection in the tick *Amblyomma americanum,* Pub Health Rep *58,* 721-729

Parker, R R, Philip, C B, and Jellison, W L, 1933, Rocky Mountain spotted fever. Potentialities of tick transmission in relation to geographical occurrence in the United States, Am J Trop Med *13,* 341-379

Parker, R R, Pickens, E G, Lackman, D B, Bell, E J, and Thrailkill, F B, 1951, Isolation and *characterization of Rocky Mountain spotted fever* rickettsiae from the rabbit tick *Haemaphysalis leporis-palustris* Packard, Pub Health Rep *66,* 455-463

[illegible] Woodward

[illegible]

Rickettsia rickettsii in a patient recovered from Rocky Mountain spotted fever, J Immunol *73,* 383-386

Patino, S, Afanador, A, and Paul, J H, 1937, A spotted fever in Tobia, Colombia Preliminary report, *Am J Trop Med 17, 639-653*

Patiño-Camargo, L, 1941, Nuevas observaciones sobre un tercer foco de fiebre petequial (maculosa) en el hemisferio americano, Bol Ofic San Panam *20, 1112-1123*

Pereira, H G, and Travassos, J, 1951, Estudo comparativo das rickettsias da febre maculosa das *Montanhas Rochosas e do "tifo de S' Paulo"* pela reação de fixação do complemento, Hospital, Rio de Janeiro *40,* 87-91

Philip, C B, 1942, Rocky Mountain spotted fever known and potential tick vectors in the United States, Proc. 6th Pacific Sc Congr *5,* 581-584

———, 1952, Tick transmission of Indian tick typhus and some related rickettsioses, Exper Parasitol *1, 129-142*

———, 1953, Nomenclature of the rickettsiaceae pathogenic to vertebrates, Ann New York Acad Sc. *56* (Art 3), 484-494

Philip, C B, Bell, J F, and Larson, C L, *1955,* Evidence of infectious diseases and parasites in a peak population of black-tailed jack rabbits in Nevada, J. Wildlife Management *19,* 225-233

Philip, C. B., and Hughes, L E, *1953,* Disease agents found in the rabbit tick, *Dermacentor parumapertus,* in the southwestern United States *in* Sixth

Internat. Congress Microbiol, Section XVI, *5*, 541-548

Philip, C B, and Kohls, G M, 1951, Elk, winter ticks, and Rocky Mountain spotted fever a query, Pub Health Rep *66*, 1672-1675

Philip, C B, and Parker, R R, 1938, The persistence of the viruses of endemic (murine) typhus, Rocky Mountain spotted fever, and boutonneuse fever in tissues of experimental animals, Pub Health Rep *53*, 1246-1251

Pijper, A, and Crocker, C G, 1938, Rickettsioses of South Africa, South African M J *12*, 613-630

Pincoffs, M C, Guy, E G, Lister, L M, Woodward, T E, and Smadel, J. E, 1948, The treatment of Rocky Mountain spotted fever with chloromycetin, Ann Int Med *29*, 656-663

Pinkerton, H., 1936, Criteria for the accurate classification of the rickettsial diseases (rickettsioses) and of their etiological agents, Parasitology *28*, 172-189

Pinkerton, H, and Hass, G M., 1932, Spotted fever I Intranuclear rickettsiae in spotted fever studied in tissue culture, J Exper Med *56*, 151-156

Piza, J, Salles-Gomes, F, Salles-Gomes, L, Meyer, J, Fleury, J P, Castro, O, Rodrigues, C, and Lima, H R., 1931, Le typhus exanthématique à Sao Paulo, Compt Rend Soc Biol *106*, 1020-1022

Plotz, H, 1945 The significance of the serologic reactions in the diagnosis of rickettsial diseases *in* Primera Reunion Interamericana del tifo, Mexico, 1945 [Proceedings of the Conference], pp 149-168, Mexico, October 1947

Plotz, H, Reagan, [redacted] L, and Wertman, K, 1944, Differentiation between Fièvre Boutonneuse and Rocky Mountain spotted fever by means of complement-fixation, Proc Soc Exper Biol & Med *55*, 173-176

Plotz, H, Smadel, J E, Bennett, B L, Reagan, R L, and Snyder, M J, 1946, North Queensland tick typhus studies of the aetiological agent and its relation to other rickettsial diseases, M J Australia, *2*, 263-268

Pope, J H, 1955, The isolation of a rickettsia resembling Rickettsia australis in south-east Queensland, M J Australia, *1*, 761-763

Powell, A M, Snyder, M J, Minor, J V, Jr, Benson, J F, and Woodward, T E, 1951, The use of Terramycin in Rocky Mountain spotted fever, Bull Johns Hopkins Hosp *89*, 30-38

Price, W H, 1953, Interference phenomenon in animal infections with rickettsiae of Rocky Mountain spotted fever, Proc Soc Exper Biol & Med *82*, 180-184

———, 1954, The epidemiology of Rocky Mountain spotted fever II Studies on the biological survival mechanism of *Rickettsia rickettsii*, Am J Hyg *60*, 292-319

Price, W H, Johnson, J W, Emerson, H, and Preston, C E, 1954, Rickettsial-interference phenomenon a new protective mechanism, Science *120*, 457-459

Puffer, R, 1957, Personal communication, Epidemiology and statistics section, Pan-American Sanitary Bureau, Washington, D C

Rao, K N A, 1951, A case of tick typhus in Srinagar, Indian J M Res *39*, 293-296

Ravenel, [redacted] F, 1947, Para-aminobenzoic acid therapy of Rocky Mountain spotted fever Outline of a comprehensive plan of treatment with report of five cases J A M A *133*, 989-994

Rose, H M, 1949, The clinical manifestations and laboratory diagnosis of rickettsialpox, Ann Int Med *31*, 871-883

———, 1950, The experimental and clinical evaluation of Terramycin against *Rickettsia akari* (Rickettsialpox), Ann New York Acad Sc *53*, 385-394

———, 1952, The treatment of rickettsialpox with antibiotics, Ann New York Acad Sc *55* (Art 6), 1019-1026

Rose, H M, Duane, [redacted] B, and Fischel, E E, 1945, The treatment of spotted fever with para-aminobenzoic acid, J A M A *129*, 1160-1161

Rose, H M, and Kneeland, Y, Jr, 1949, Aureomycin in the treatment of infectious diseases, Am J Med *7*, 532-541

Rosenblum, M J, Masland, R L, and Harrell, G T, 1952, Residual effects of rickettsial disease on the central nervous system Results of neurologic examinations and electroencephalograms following Rocky Mountain spotted fever A M A Arch Int Med *90*, 444-445

Ross, S, Schoenbach, E B, Burke, F G, Bryer, M S, Rice, E C, and Washington, J A, 1948, Aureomycin therapy of Rocky Mountain spotted fever, J A M A *138*, 1213-1216

Rumreich, A, Dyer, R E, and Badger, L F, 1931, The typhus-Rocky Mountain spotted fever group, Pub Health Rep *46*, 470-480

Schubert, J H, 1952, Serologic titers in rickettsial infection as affected by antibiotic treatment, Pub Health Lab *10*, 38-41

Schubert, J H, Stanford, S M, and Tiffany, E J, 1951, Comparative evaluation of several complement fixation techniques for laboratory diagnosis of the rickettsioses, J Lab & Clin Med *37*, 388-393

Scientific Session Summaries Academy of Medical Science of the U S S R and the Ministry of Public Health of the Uzbek [redacted] S R, 1954, Scientific session on problems of regional pathology Sept 20-25 (Translations through courtesy of Dr Karl F Meyer, San Francisco, California.)

Seaton, D R, and Stoker, M [redacted] P, 1946, A serological analysis of typhus cases in India by Weil-Felix, rickettsial agglutination and complement-fixation tests, Ann Trop Med *40*, 347-357

Shankman, B, 1946, Report on an outbreak of endemic febrile illness not yet identified, occurring in New York City, New York J Med *44*, 2156-2159

Silva-Goytia, R, and Elizondo, A, 1952, Estudios sobre fiebre manchada en Mexico [illegible]

Smadel, J E, 1951, Present status of antibiotic therapy in viral and rickettsial disease, Bull New York Acad Med *27*, 221-231

Smadel, J E, and Jackson, E B, 1947, Chloromycetin, an antibiotic with chemotherapeutic activity in experimental rickettsial and viral infections, Science *106*, 418-419

Smadel, J E, Jackson, E B, and Cruise, A B, 1949, Chloromycetin in experimental rickettsial infections, J Immunol *62*, 49-65

Smadel, J E, Jackson, E B, and Ley, H L, Jr, 1950, Terramycin as a rickettsiostatic agent and its usefulness in patients with scrub typhus, Ann New York Acad Sc *53*, 375-384

Smith, C N, Cole, M M, and Gouck, H K, 1946, Biology and control of the American dog tick, U S Dept of Agric, Tech Bull 905, 74 pp, U S Gov't Printing Office, Washington, D C

Snyder, J C, Fagan, R, Wells, E B, Wick, H C, and Miller, J C, 1950, Experimental studies on the antirickettsial properties of Terramycin, Ann New York Acad Sc *53*, 362-374

Snyder, J C, Maier, J, and Anderson, C R, 1942, Report to the Division of Medical Sciences, National Research Council, Dec 26

Soman, D W, 1954, Rickettsial diseases in India Their laboratory and experimental study Haffkine Institute, Bombay Printed at the Government Central Press, Bombay

Spencer, R R, and Parker, R R, 1923, Studies on Rocky Mountain spotted fever infectivity of fasting and recently fed ticks, Pub Health Rep *38*, 333-339

———, 1930, Infection by other means than the tick bite *in* Studies on Rocky Mountain Spotted Fever, Nat Inst Health Bull *154*, 116 pp

Steinhaus, E A, 1946 Insect Microbiology, 763 pp, Ithaca, N Y, Comstock

Streeten, G E W, Cohen, R S, Gutteridge, N M, Wilmer, N B, Brown, H E, Smith, D J W, and Derrick, E H, 1948, Tick typhus in South Queensland, report of 3 cases, M J Australia *1*, 372-373

Sussman, L N, 1946, Kew Gardens' spotted fever, New York Med *2*, (15) 27-28

Therrien, A A, Hunter, G W, III, Moon, A P, and Adams, A L, 1954, Tests of potential acaricides against the lone star tick, J Econ Entomol *47*, 76-78

Topping, N H, 1941, Rocky Mountain spotted fever A note on some aspects of its epidemiology Pub Health Rep *56*, 1699-1703

———, 1943, Rocky Mountain spotted fever Further experience in the therapeutic use of immune rabbit serum, Pub Health Rep *58*, 757-775

Topping, N H, Heilig, R, and Naidu, V R, 1943, A note on the rickettsioses in India, Pub Health Rep *58*, 1208-1210

Travassos, J, 1948 Studies on rickettsial diseases in Brazil *in* Whitlock, C, (ed) Proceedings of the Fourth international congresses on Tropical Medicine and Malaria, Vol 1, pp 414-421, Washington, D C, U S Government Printing Office

van der Scheer, J, Bohnel, E, and Cox, H R, 1947, Diagnostic antigens for epidemic typhus, murine typhus and Rocky Mountain spotted fever, J Immunol *56*, 365-375

Wolbach, S B, 1948 The pathology of the rickettsial diseases of man *in* Moulton, F R, (ed) Rickettsial Diseases of Man, pp 118-125, Washington, D C, Am Assoc Advancement Sc

Wong, S C, and Cox, H R, 1948, Action of Aureomycin against experimental rickettsial and viral infections, Ann New York Acad Sc *51*, 290-305

Wood, W M, 1896, Annual Report of the Surgeon General to the Secretary of War

Workman, J B, Hightower, J A, Borges, F J, Furman, J E, and Parker, R R, 1952, Cortisone as an adjunct to chloramphenicol in the treatment of Rocky Mountain spotted fever, New England J Med *246*, 962-966

Yanez, R, 1957, Therapeutic action of Achromycin in the treatment of exanthematous typhus fever Personal communication to Dr F C Ottati, American Cyanamid Company, Pearl River, N Y

Yeo, R M, 1950, Common tropical diseases encountered at the W N L A and their treatment, with special reference to some of the newer drugs, Proc Transvaal Mine M Officers' Assn *29* (317), 93-102

Zinsser, H, FitzPatrick, F, and Wei, H, 1939, A study of rickettsiae grown on agar tissue cultures, J Exper Med *69*, 179-190

Zdrodovsky, P F, and Golinevich, Y M, 1953 The pathogenic rickettsia, general characteristics and systematic classification of the rickettsioses in A Study of Rickettsiae and Rickettsioses, ed 1, pp 7-22, Moscow, Medgiz (Translation courtesy of Dr C B Philip, Rocky Mountain Laboratory, Hamilton, Montana)

———, 1956a, Tick-bite rickettsioses or tick-borne spotted fever in north Asia (Ixodo-rickettsiosis Asiatica) *in* A Study of Rickettsiae and Rickettsioses, ed 2, pp 254-271, Moscow, Medgiz (Translations courtesy of Dr J E Smadel, Nat'l Inst of Health, Washington, D C, and Mr F Danga, American Cyanamid Company, Pearl River, N Y)

———, 1956b Vesicular and smallpox-like rickettsioses *in* A Study of Rickettsiae and Rickettsioses, ed 2, pp 276-286, Moscow, Medgiz (Translations through courtesy of Dr J E Smadel, Nat'l Inst of Health, Washington, D C, and Mr F Danga, American Cyanamid Company, Pearl River, N Y)

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44

Scrub Typhus

(SYNONYMS. Tsutsugamushi disease, mite-borne typhus, Japanese river fever, tropical typhus, rural typhus)

INTRODUCTION

Scrub typhus is an infectious disease caused by *Rickettsia tsutsugamushi* and characterized by sudden onset, fever of about 2 weeks' duration and a cutaneous rash which appears on approximately the 5th day The disease is transmitted by certain mites, and an eschar usually develops at the site of attachment of the "chigger " Patients with scrub typhus generally develop agglutinins against the OX-K strain of proteus bacillus Specific antibiotic therapy controls scrub typhus

HISTORY

Scrub typhus was described almost 150 years ago in Japan, and during the past 75 years has been studied assiduously by investigators of that country who discovered the vector, the etiologic agent and the rodent reservoir (cf Kawamura, 1926, Sasa, 1954) Gradually, the disease was recognized in other areas and now is known to be widespread in the roughly triangular region bounded by Japan, India and Australia (Blake et al, 1945) During World War II scrub typhus was of considerable military importance to both Allied and Japanese forces operating in the Southwest Pacific area and in the China-Burma-India Theater. Approximately 18,000 cases occurred among Allied soldiers, and in some instances the explosive outbreaks were devastating For example, 403 men in an American regiment developed the disease between the 6th and the 20th days after landing at Sansapor, moreover, the attack rate during the 2nd week at Sansapor reached 900 cases per 1,000 troops per year The fatality rates varied in epidemics in different areas, thus among the 6,685 U S Army personnel afflicted with scrub typhus, the mortality rates were as low as 0 6 per cent in some regions and as high as 35 3 per cent in others Effective measures for controlling the mite vector were developed by investigators in America and the British Commonwealth When these were applied, scrub typhus ceased to be a disease of great military importance (Philip, 1948, Fairley, 1951).

CLINICAL PICTURE

Following an incubation period of from 6 to 21 days, generally 10 to 12, illness begins suddenly with fever, chilliness, severe headache, conjunctival injection and moderate generalized lymphadenopathy. The primary lesion or eschar is found in the majority of Caucasians but in few Asiatics It occurs at sites where skin surfaces meet or clothes bind, viz, axilla and waist At the onset of fever the eschar is an indurated erythematous lesion about 1 cm in diameter surmounted by a multiloculated vesicle Within a few days the vesicle ulcerates, and the area is cov-

ered by a black scab; the regional lymph nodes become particularly prominent. The fever increases progressively during the first week of the disease, generally reaching 104° or 105° F. The pulse rate during this period is relatively slow, being 70 to 100. A red macular rash appears on the trunk between the 5th and the 8th days and may extend to the arms and the legs. The macular eruption usually persists for several days but may become maculopapular; contrariwise, it may disappear at times within a few hours.

During the first week of fever, cough is commonly present Physical and roentgenographic evidence of pneumonitis occurs frequently; Ahlm and Lipshutz (1944) observed the former in 67 per cent and the latter in 20 per cent of 70 patients

The temperature remains elevated during the 2nd week in patients who do not receive specific therapy Headache may abate somewhat after the 7th day, but apathy continues, and some deafness is commonly present. Certain patients develop additional signs of involvement of the central nervous system, for example, delirium, stupor and muscular twitching In the more severely affected patients the pulse rate increases to 120 or 140, and the systolic blood pressure may fall below 100 Frank signs of pneumonia or of circulatory failure develop in some patients Toward the end of the 2nd week or the beginning of the 3rd, the temperature of the untreated patient who is destined to recover falls by lysis over a period of several days With the reduction in fever, the pulse rate and blood pressure return to normal, the eschar is practically healed, and the spleen is no longer palpable if it had been felt during the febrile period Convalescence is generally protracted in those in whom the disease has run its full course Sequelae in the form of nervous or psychiatric difficulties were frequent among military patients, it is probable that the rigorous campaigns and intercurrent [illegible] con- [illegible] ne [illegible] d in some of the patients, might proceed to permanent damage of the heart has been overemphasized. When specific antibiotic therapy is employed during the 1st week of illness complications and sequelae are avoided, and convalescence is short

Death, when it occurs, supervenes about the end of the 2nd week and is attributable in approximately equal numbers of cases to secondary bacterial pneumonia, encephalitis or circulatory failure. The mortality varies from 1 to 60 per cent in different geographic areas and different populations. Since the introduction of specific antibiotic therapy in 1948, the picture has changed radically, and now the mortality approaches zero

Second attacks of scrub typhus are not uncommon and may occur in exposed persons within a few years after the initial illness Observations on naturally acquired disease have been augmented by studies on volunteers (Smadel et al, 1950b). Antigenic differences in strains of *R. tsutsugamushi* play an important role in susceptibility to reinfection Persons recovered from illness caused by one strain are resistant to this strain for a number of years and to heterologous strains for some months. However, the majority of such persons are susceptible to infection with heterologous strains 1 to 2 years after recovery. In some patients *R. tsutsugamushi* persists for many months after apparent recovery. This organism was isolated from a lymph node removed from one individual more than a year after infection, but similar attempts in 11 other instances yielded negative results (Smadel et al., 1952). The occasional person who is immune to a heterologous strain some years after a first attack may be one in whose tissues rickettsiae persisted and constantly stimulated the immune mechanism Recurrent scrub typhus analogous to Brill-Zinsser disease, which is a recurrence of epidemic typhus years after the original disease (cf. Chap 42), has not been recognized. However, the demonstrated persistence of *R. tsutsugamushi* in some recovered patients sets the stage for such a possibility and warrants a continuing search for late recurrences of scrub typhus.

There is no specific blood picture in scrub typhus. The leukocyte count remains essentially within the normal range unless secondary bacterial infection occurs. Anemia is rarely observed. Plasma proteins may be lowered slightly during the febrile illness If this occurs, the proportionately greater decrease

in albumin content of the plasma may produce a reversal of the albumin-globulin ratio Hypochloremia often develops late in the febrile stage as a result of inadequate salt intake and excessive sweating Certain patients with hepatic impairment show a decrease in plasma fibrinogen and an elevated icteric index.

PATHOLOGIC PICTURE

Changes observed at necropsy are not striking. Usually the eschar is found, but no rash

a superimposed, secondary bronchopneumonia The spleen and lymph nodes are enlarged

Microscopic examination brings out the fact that here, as in other rickettsial diseases, the vascular tree is primarily affected, showing a disseminated focal vasculitis and a perivasculitis of the smaller vessels consisting of accumulations of monocytes, plasma cells and lymphocytes These lesions are less severe than in epidemic typhus, furthermore, the necrosis and inflammatory reaction of the vessel wall, so characteristic of spotted fever, if present, are limited to the eschar. Vascular changes with resultant lesions in adjacent parenchymatous tissue are most conspicuous in heart, lung, brain and kidney Thus, an acute, nonsuppurative myocarditis of focal and diffuse distribution and of varying intensity is characteristically present Interstitial pneumonitis occurs in practically all fatal cases The lesions in the brain may consist of a few vascular and perivascular reactions such as are found throughout the body In certain instances, however, a true lymphocytic meningitis and an encephalitis with perivascular cuffing and formation of glial nodules occur The spleen and the lymph nodes display similar changes with infiltra-

are associated with damage to adjacent nephrons

EXPERIMENTAL INFECTION, HOST RANGE

The host range of *R tsutsugamushi* is broad, several species of mites, many species of rodents (cf. Epidemiology), and man are infected in nature, and a number of the common laboratory animals are susceptible White mice are the animals of choice for most laboratory studies of *R. tsutsugamushi.* Seven or 8 days after intraperitoneal inoculation of highly infectious material the mouse appears sick; during the next few days the abdomen swells, subcutaneous edema of the abdominal wall may appear, dyspnea develops, and death ensues. At necropsy, in addition to subcutaneous edema, lymphadenitis is apparent when the skin is reflected, the peritoneal cavity contains several cubic centimeters of serofibrinous exudate, and the surfaces are injected; finally, the spleen is enlarged and usually is coated with flecks of fibrinous ex-

ettsiae can be found in impression smears of any of the involved tissues but are demonstrated most readily in stained smears prepared from the surface of the spleen or the peritoneum. Blood, exudates and all tissues are infectious Suspensions of splenic tissue from mice infected with one of the typical laboratory strains of *R tsutsugamushi* usually have a lethal titer of about 10^{-7} when inoculated into mice by the intraperitoneal route, the titer of the blood from such animals is about 10^{-5}. Mice become infected with scrub typhus following inoculation by the subcutaneous, the intranasal, or the intravenous route, but the resultant disease differs somewhat from that described above For example, the lethal titer of a suspension of rickettsiae may be 10^{-7} when tested intraperitoneally but only 10^{-2} on subcutaneous inoculation; nevertheless, a 10^{-5} dilution of the same suspension administered subcutaneously induces an inapparent infection which is followed by immunity. The intravenous or intranasal inoculation of mice with suspensions rich in organisms results in their death in 4 to 5 days with hemorrhagic lesions in their lungs, suspensions of such pulmonary tissue have titers of 10^{-8} to 10^{-9}. Strains of *R. tsutsugamushi* vary widely in their virulence for mice; with some the minimal lethal dose and the minimal infectious dose are practically identical when the intraperitoneal route is used, while with others the lethal dose may

ered by a black scab; the regional lymph nodes become particularly prominent. The fever increases progressively during the first week of the disease, generally reaching 104° or 105° F. The pulse rate during this period is relatively slow, being 70 to 100 A red macular rash appears on the trunk between the 5th and the 8th days and may extend to the arms and the legs The macular eruption usually persists for several days but may become maculopapular, contrariwise, it may disappear at times within a few hours

During the first week of fever, cough is commonly present. Physical and roentgenographic evidence of pneumonitis occurs frequently, Ahlm and Lipshutz (1944) observed the former in 67 per cent and the latter in 20 per cent of 70 patients

The temperature remains elevated during the 2nd week in patients who do not receive specific therapy. Headache may abate somewhat after the 7th day, but apathy continues, and some deafness is commonly present. Certain patients develop additional signs of involvement of the central nervous system, for example, delirium, stupor and muscular twitching. In the more severely affected patients the pulse rate increases to 120 or 140, and the systolic blood pressure may fall below 100. Frank signs of pneumonia or of circulatory failure develop in some patients Toward the end of the 2nd week or the beginning of the 3rd, the temperature of the untreated patient who is destined to recover falls by lysis over a period of several days With the reduction in fever, the pulse rate and blood pressure return to normal, the eschar is practically healed, and the spleen is no longer palpable if it had been felt during the febrile period Convalescence is generally protracted in those in whom the disease has run its full course Sequelae in the form of nervous or psychiatric difficulties were frequent among military patients, it is probable that the rigorous campaigns and intercurrent diseases which accompanied scrub typhus contributed materially to this high incidence The possibility that myocarditis, which occurred in some of the patients, might proceed to permanent damage of the heart has been overemphasized When specific antibiotic therapy is employed during the 1st week of illness complications and sequelae are avoided, and convalescence is short.

Death, when it occurs, supervenes about the end of the 2nd week and is attributable in approximately equal numbers of cases to secondary bacterial pneumonia, encephalitis or circulatory failure The mortality varies from 1 to 60 per cent in different geographic areas and different populations. Since the introduction of specific antibiotic therapy in 1948, the picture has changed radically, and now the mortality approaches zero.

Second attacks of scrub typhus are not uncommon and may occur in exposed persons within a few years after the initial illness Observations on naturally acquired disease have been augmented by studies on volunteers (Smadel et al., 1950b) Antigenic differences in strains of *R. tsutsugamushi* play an important role in susceptibility to reinfection Persons recovered from illness caused by one strain are resistant to this strain for a number of years and to heterologous strains for some months However, the majority of such persons are susceptible to infection with heterologous strains 1 to 2 years after recovery. In some patients *R. tsutsugamushi* persists for many months after apparent recovery. This organism was isolated from a lymph node removed from one individual more than a year after infection, but similar attempts in 11 other instances yielded negative results (Smadel et al, 1952). The occasional person who is immune to a heterologous strain some years after a first attack may be one in whose tissues rickettsiae persisted and constantly stimulated the immune mechanism Recurrent scrub typhus analogous to Brill-Zinsser disease, which is a recurrence of epidemic typhus years after the original disease (cf. Chap 42), has not been recognized. However, the demonstrated persistence of *R tsutsugamushi* in some recovered patients sets the stage for such a possibility and warrants a continuing search for late recurrences of scrub typhus

There is no specific blood picture in scrub typhus. The leukocyte count remains essentially within the normal range unless secondary bacterial infection occurs. Anemia is rarely observed Plasma proteins may be lowered slightly during the febrile illness If this occurs, the proportionately greater decrease

FIG 131 Electronmicrograph of a thin section of an MB tissue culture cell infected with *R tsutsugamushi* (×40,000) The rickettsiae appear as large rodlike structures in a portion of the cytoplasm which contains normal endoplasmic reticulum and organelles The external limiting membranes of the rickettsiae are clearly discernible, as are granular structures within the organism (Prepared by S L Wissig and L G Caro by methods described in Wissig, et al, 1956)

strain of *R tsutsugamushi* are protected from disease (Topping, 1945) The neutralization technic has been useful in studies on the antigenic variations in different strains of *R tsutsugamushi* (Bell et al, 1946, Bennett et al, 1947, Fox, 1949) The extent of the antigenic diversity of strains collected in a small area is illustrated by the experience of Miesse et al (1950) Eight strains, recovered from volunteers exposed in a field of several hundred acres and from mites collected on rodents inhabiting the field, fell into 4 serogroups, and only one of these was closely related to a strain of *R tsutsugamushi* previously characterized by means of extensive cross-neutralization tests Such antigenic heterogeneity undoubtedly contributes to the susceptibility of human beings to second attacks of scrub typhus as well as to the difficulties encountered in preparing a vaccine which will protect man against exposure under field conditions (see below)

A toxin is associated with the organism of scrub typhus (Smadel et al, 1946a, Cox, 1951), its properties are similar to those of other rickettsial toxins (cf Chap. 10). Embryonated eggs infected with certain strains of *R. tsutsugamushi* provide a material which kills mice within a few hours Antitoxin capable of neutralizing this substance appears in the sera of animals convalescent from infection with the homologous strain and in the sera of some patients However, sera drawn from animals following infection with heterologous strains of *R tsutsugamushi* may not contain antitoxin, even though homologous neutralizing and complement-fixing antibodies are present

During and after World War II intensive efforts were made to develop a vaccine for scrub typhus Several groups of investigators prepared noninfectious materials which were capable of inducing appreciable resistance in laboratory animals to infection with the homologous strain of *R. tsutsugamushi* Such vaccines were derived from infected tissue obtained from cotton rats (Fulton and Joyner, 1945), white rats (Smadel et al., 1946b), agar

be *100 to 1000* times greater than the infectious dose (Jackson and Smadel, 1951); finally, certain of the strains are rarely lethal for mice under any condition Such variations in virulence have been noted among recently isolated strains and also have been observed to develop in strains maintained in the laboratory Immune mice for use in cross protection tests are obtained most readily by treating infected animals with chloramphenicol For this, mice which have been injected by the intraperitoneal route with 100 to 1000 lethal doses of the immunizing strain are provided with drinking water containing 2.5 mg of chloramphenicol per ml for a period of 3 weeks (Jackson et al., 1957).

Rickettsia tsutsugamushi grows well in the yolk-sac tissue of embryonated eggs and in several types of tissue cultures Five- or 6-day-old chick embryos are inoculated into the yolk sac with highly infectious material and then incubated at 35° C Death occurs in 6 to 10 days without pathognomonic lesions Rickettsiae are most numerous in the yolk-sac tissue, which has an infectious titer of 10^{-8} to 10^{-9}, and are readily demonstrable in stained smears Infected yolk-sac tissue serves as seed inoculum, as a source of the toxin of scrub typhus, and as starting material for the preparation of rickettsial complement-fixing antigens

Cotton rats infected by the intranasal route and white rats injected intravenously with highly infectious inocula die in 4 to 6 days and yield lung tissue with infective titers of 10^{-8} to 10^{-9} Studies on the experimental disease in monkeys, rabbits and guinea pigs provided information of historic importance, but such animals are not employed extensively at present; in the monograph by Blake et al. (1945) these studies are reviewed, as well as early work on growth of the agent in tissue culture and the experimental disease in hamsters Several species of gerbilles (rodents native to Africa) are susceptible to infection with *R tsutsugamushi* and have proved to be useful in certain types of studies (Murray et al, 1945; Mackie et al, 1946).

ETIOLOGY

The etiologic agent of scrub typhus has the general properties of rickettsiae The obligate, intracellular, parasitic, pleomorphic micro-organisms are usually seen as small diplococcuslike structures or as short rods, with bipolar dark-staining bodies, which have a length of from 0.3 to 0 5 μ and a width of from 0 2 to 0 4 μ. The rickettsiae appear as purple structures in the cytoplasm of cells when viewed in impression smears of infected tissues stained by Giemsa's method The morphologic structure of *R. tsutsugamushi* as revealed by electronmicroscopy is similar to that of other rickettsiae, the organism has a limiting membrane enclosing protoplasmic material in which are dispersed dense granules (Wissig et al, 1956), shown in Figure 131 This rickettsia multiplies at a slow rate in comparison with viruses and bacteria; thus, it increases about 3-fold each 24 hours when growing in the MBIII strain of cultured mouse cells (Bozeman et al, 1956).

R. tsutsugamushi is relatively labile, 10 per cent suspensions of infected tissues freed of large particles by light centrifugation are rendered noninfectious within a few hours by the addition of 0 1 per cent U S P formaldehyde solution. However, the organism remains viable for long periods of time when stored at −70° C in whole tissues or suspended in various protective media (Bovarnick et al, 1950) Lyophilization under carefully controlled conditions is relatively satisfactory (Jackson and Smadel, 1951), nevertheless, transportation of a newly isolated strain from the field to a central laboratory is accomplished best by shipment of infected mice if dry-ice refrigeration is not available

Specific complement-fixing antigens of scrub typhus have been prepared from several types of infected material, namely, yolk-sac tissue, mouse lung, white rat lung and cotton rat lung Methods, in which ether extraction of undiluted ground infected yolk-sac tissue is used, have provided satisfactory scrub typhus antigens, the author prefers the procedure of Topping and Shepard, method C (1946) Such antigens are of some value in the serodiagnosis of the disease in patients (cf. Diagnosis).

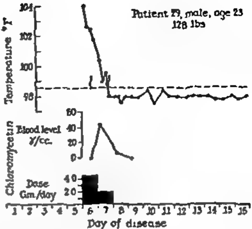
In scrub typhus, as in other viral and rickettsial diseases, convalescent animals and patients develop specific neutralizing antibodies which are detectable in their sera Mice inoculated with such sera and the homologous

as diagnostic procedures. Hence, the nonspecific Weil-Felix reaction continues to be the most useful serologic test for the diagnosis of scrub typhus in man. Agglutinins for the OX-K strain of *B. proteus* generally appear in a patient's serum by the end of the 2nd week, but none develops against the OX-19

mens of serum be obtained to demonstrate the appearance and the rise in titer of OX-K agglutinins. While a titer of 1/160 obtained with a single convalescent serum is generally regarded as significant, the result of one Weil-

in scrub typhus Indeed, in a fair number of instances patients with scrub typhus fail to develop OX-K titers of 1/160, although serial examinations may show a diagnostic 4-fold rise in the agglutinins It should be recalled that relapsing fever may evoke an OX-K response similar to that displayed in scrub typhus (Zarafonetis et al, 1946).

In evaluating the laboratory diagnostic procedures employed in scrub typhus Diercks (1951) analyzed the results obtained in 124 Malayan patients with the disease. In each instance the clinical diagnosis was confirmed by one or another of the 3 tests employed *R tsutsugamushi* was isolated from 92 per cent of the cases Although 70 per cent of the patients displayed a rise in OX-K agglutinins, only 52 per cent developed antibodies during convalescence which fixed complement in the presence of soluble antigen prepared from heavily infected yolk sacs Thus, a positive complement-fixation test is of diagnostic significance, since the reaction is specific However, a negative result is of no value in eliminating the diagnosis of scrub typhus. Doherty (1956) in a somewhat similar study found that "for the practical purpose of diagnosing scrub typhus in North Queensland the Weil-Felix reaction can be expected to detect only a little more than half the cases that occur" In the last analysis then, isolation of *R tsutsugamushi* in mice inoculated with blood from a febrile patient is the most reliable of



Rash	++ + 0
Eschar	+ + + + ± 0
W F OX-K	80 320
Rickettsemia	+ + + 0

FIG 132. Response of scrub typhus Patient 29 to chloramphenicol. (Smadel, et al., 1949, Chloramphenicol [Chloromycetin] in the treatment of tsutsugamushi disease [scrub typhus], J Clin Invest. *28*, 1202)

any single test, while the Weil-Felix is the simplest to perform

TREATMENT

Chloramphenicol, chlortetracycline and oxytetracycline are specific therapeutic agents for scrub typhus Patients are rendered afebrile and almost asymptomatic in a day or two after institution of therapy Figure 132 illustrates the prompt return of temperature to normal levels within 24 hours following administration of chloramphenicol to one of the patients in the first group studied (Smadel et al, 1949)

Each of the specific antibiotics may be

tered over a period of 24 hours is adequate, but in the more seriously ill patients treatment may be required for another day or two There are no ordinary contraindications to the use of these highly effective drugs in this

tissue cultures (Plotz et al., 1946) and embryonated eggs (Bailey et al., 1948). None of the three field trials in which killed scrub typhus vaccines were employed gave encouraging results (Card and Walker, 1947; Berge et al., 1949). It is noteworthy that one of these tests was made with Malayan volunteers who were exposed by sitting in the grass in a hyperendemic area. These men had received a vaccine containing a strain of rickettsia which had been recovered earlier from infected mites collected from the exposure-area used for challenge (Smadel et al., 1950a). Such failures led the author to abandon studies on inactive scrub typhus vaccines and to turn to chemoprophylaxis of exposed persons and to the use of live vaccine combined with chemoprophylaxis to suppress clinical disease without interfering with the development of immunity. These last procedures, which were reasonably successful, are discussed below (cf Control Measures).

Chemotherapeutic studies on *R. tsutsugamushi* have been closely connected with those on other rickettsiae The steps which led to the great success in treatment of these infections have been reviewed elsewhere (Snyder, 1948; Smadel, 1949) Suffice it to say that the first significant development in the therapeutic conquest of scrub typhus began with the demonstration of Snyder et al (1942) that para-aminobenzoic acid possessed antirickettsial activity in mice infected with *R typhi*. This was followed in 1947 by the observation that chloramphenicol was highly effective in treating mice with experimental scrub typhus (Smadel and Jackson, 1947). The therapeutic value of the antibiotics chloramphenicol, chlortetracycline and oxytetracycline in patients with this disease is elaborated on below (cf. Treatment) Jackson (1951), who reviewed current experimental work and added her own comparative studies, concluded. "The general order of rickettsiostatic activity of eight substances as

cillin G, subtilin and streptomycin . . . Subtilin is more effective against *R. tsutsugamushi* than against *R. typhi* or *R. rickettsii*, while the reverse order is true for PABA." It must be emphasized that none of the clinically useful drugs is rickettsiocidal in the concentrations attained in a patient's blood; each is active because of its rickettsiostatic properties.

DIAGNOSIS

A history of exposure in an area where scrub typhus is endemic and the finding of the primary lesion (eschar) are of great assistance in the early diagnosis of the disease. Additional signs and symptoms of the malady, such as headache, conjunctival injection, fever, relative bradycardia and absence of leukocytosis, are common to many diseases which occur in scrub typhus areas, for example, other rickettsial infections, dengue, malaria, infectious hepatitis, and typhoid fever. The appearance of the skin eruption at about the end of the first week of fever is of some diagnostic assistance.

A specific diagnosis of scrub typhus may be made by recovering the causal rickettsia from the blood of a patient during the febrile period or from tissues obtained at necropsy (Smadel, 1956). A suspension of ground blood clot or of tissue is injected intraperitoneally into white mice Infected mice may die from 10 to 18 days after inoculation and show serofibrinous peritonitis and enlarged spleens. Microscopic examination of impression smears made from the surface of the spleen or the parietal peritoneum, fixed in methyl alcohol and stained by Giemsa's method, reveal the presence of minute intracellular and extracellular diplococcal organisms which have a purple color. The experimental disease is maintained by passage of bacteriologically sterile peritoneal fluid, blood, or suspensions of spleen, liver or lung Final identification of the rickettsial agent is made by cross-immunity tests performed in mice. Not all strains of *R. tsutsugamushi* are lethal for mice when first recovered from patients Therefore, in some instances examination and passage of materials from sick or apparently healthy animals may be necessary to establish the strain.

Specific serologic tests in which rickettsial materials are employed are of limited value

exacting conditions necessary for a focus result in such a sharp perimeter that Audy and Harrison (1951) have called the areas "typhus islands" Thus, in the endemic areas in Japan infection may be contracted in an uncultivated area on the river side of an embankment but not in the tilled field just inside the dike Recently, scrub typhus has been recognized among inhabitants of certain Japanese islands during the autumn and the winter when the classic vectors are absent *Trombicula scutellaris* from these islands was found infected with *R. tsutsugamushi* and on epidemiologic and ecologic grounds appears to be the vector in this region (Sasa, 1954) Elsewhere in Japan and also in Korea, *T pallida* is infected in nature and appears to be the common vector for transmission of the organism to rodents and occasionally to man (Jackson et al, 1957)

CONTROL MEASURES

The prevention of scrub typhus during military campaigns has been attained by the application of control measures aimed at the mite vector, these consist of the use of insecticides by the individual and of appropriate treatment of the terrain of endemic areas (Department of the Army Technical Bulletin TB Med 31, Scrub Typhus, 1954) These procedures cannot be expected to provide such satisfactory results when used by civilians in peacetime because of their cost and the difficulties associated with their attainment Measures for the individual are built around the use of miticidal chemicals, such as dimethyl phthalate, dibutyl phthalate, and benzyl benzoate which are applied to the clothing either by hand or by dipping In addition, insect repellent fluid containing phthalate is applied to the exposed skin The systematic use of such procedures undoubtedly reduces greatly the incidence of scrub typhus in exposed susceptible persons, but it is difficult to obtain statistically significant data on this point However, Fairley (1951) in speaking of the control program said that " within one year of its introduction in New Guinea the rate had fallen in infected areas from 36 per 1,000 to approximately 1 per 1,000" The method employed for disinfecting a camp site is as follows All vegetation is cut level with the ground and burned or hauled away. After thorough clearing, the ground usually dries sufficiently in 2 or 3 weeks to kill the mites. If the site is to be occupied before this time, the ground may be sprayed with a miticide, such as dieldrin or lindane, which will render the area free of mites for several weeks. The prevention of scrub typhus, like that of malaria, can be accomplished by control of the insect vector, however, the cost and the effort in each instance is so great that the measures cannot be employed except by an enlightened and wealthy society.

Chemoprophylaxis with chloramphenicol has been used successfully in suppressing scrub typhus in Malayan volunteers exposed in hyperinfected areas Administration of 3-Gm oral doses at weekly intervals controlled infection sufficiently to permit volunteers to remain ambulatory even though rickettsemia occurred from time to time If the drug was continued for approximately a month after the infecting mite-bite, the person remained well, but shorter periods of chemoprophylaxis were followed by clinical disease within a week after the last dose of drug (Smadel et al, 1950c).

When noninfectious scrub typhus vaccines were found in field trials to be of no value (cf Etiology), a combined procedure was studied which consisted of inoculation of volunteers with a vaccine containing living rickettsiae, together with administration of chloramphenicol according to the chemoprophylactic schedule described above The volunteers developed a primary lesion at the site of inoculation and, although ambulatory, had rickettsemia and subsequently displayed the antibody response of scrub typhus fever. Here, as in the chemoprophylactic field studies, withdrawal of the antibiotic before the 3rd week was followed by clinical disease, but prolongation into the 4th week allowed time for immunity to develop, and breakthroughs did not occur (Smadel et al, 1951) This combined procedure is cumbersome and is not yet suitable for general use, but it is applicable under special circumstances

There is no evidence of communicability of scrub typhus from man to man. Therefore,

TABLE 40 SCRUB TYPHUS PATIENTS TREATED WITH CHLORAMPHENICOL, AUREOMYCIN, TERRAMYCIN OR PARA-AMINOBENZOIC ACID

THERAPY	NUMBER OF PATIENTS	AVERAGE DURATION OF FEVER AFTER TREATMENT (hrs)	FATALITIES
CHLOR-AMPHENICOL	100	31	0
AUREOMYCIN	29	25	0
TERRAMYCIN	7	47	0
PASA	15	89	0
		DURATION OF DISEASE	
NONE	19	17 DAYS	1

(From Smadel, J. E., 1951, Present status of antibiotic therapy in viral and rickettsial disease Bull New York Acad Med, *27*, 223)

serious disease The parenteral forms of these antibiotics are indicated in rare instances in which oral medication is impossible. Table 40 summarizes the results obtained by the U S. Army Scrub Typhus Research Unit in the treatment of 170 Malayan patients who had scrub typhus. The 3 antibiotics were about equally efficacious and were superior to para-aminobenzoic acid, which had been the best drug prior to the discovery of the broad-spectrum antibiotics.

The antibiotics do not sterilize the tissues but elicit their effect by suppressing growth of the rickettsiae Ultimate recovery depends on the development of immunity by the patient. The suppressive effect of the 24-hour regimens mentioned earlier lasts for about 1 week. Immunity begins to develop late in the 2nd week of illness and gains the ascendency over the agent on about the 14th day Hence, relapses are practically never seen in patients in whom treatment is begun on the 7th day of disease or later. On the other hand, recrudescence of disease is noted in about half the patients started on the short course of therapy on the 4th to the 6th day of illness and in about three quarters of those given specific antibiotic on the 2nd day after onset Relapses respond promptly to another course of 3 to 5 Gm. of antibiotic. Moreover, they can be prevented, in those patients who [illegible] be expected to relapse, by administer[illegible] single 3-Gm. dose of antibiotic on the 6t[illegible] after termination of the original cour[illegible] therapy (Smadel et al., 1950a).

Severely ill patients, who do not re[illegible] specific therapy until late in the disease, still require the general supportive mea[illegible] and good nursing care which were so in[illegible]tant prior to the introduction of the b[illegible] spectrum antibiotics. Penicillin is of no [illegible] in uncomplicated scrub typhus, and su[illegible]amides are contraindicated Patients w[illegible] disease has been terminated by therapy *ing the 1st week* after onset enjoy a r[illegible] convalescence and may return to seden[illegible] occupations within 10 days to 2 weeks a[illegible] becoming afebrile. Those with a more [illegible]tracted illness should be permitted a lo[illegible] convalescence

EPIDEMIOLOGY

Scrub typhus occurs throughout many [illegible] the islands, coastal and river areas of east Asia, as well as in India, southeast Asia [illegible] northern Australia (for references cf Ja[illegible]son et al, 1957). The disease is transmit[illegible] by the tiny larvae of several species of mit[illegible] These chiggers, after attachment to the sk[illegible] obtain a meal of tissue juices, in the course feeding they may acquire infection from [illegible] host or transmit rickettsiae to the vertebra[illegible]

The ecology of scrub typhus througho[illegible] most of the wide geographic distribution [illegible] the disease is the classic pattern elucidat[illegible] during the early part of this century by t[illegible] Japanese scientists in the Niigata prefectu[illegible] (Kawamura, 1926, Mackie et al, 1946, Au[illegible] and Harrison, 1951). This pattern encom[illegible]passes the cyclic maintenance in nature of *[illegible] tsutsugamushi* by means of small rodents a[illegible] *Trombicula akamushi* and *T deliensis* wit[illegible] transmission to man during periods of chigg[illegible] abundance, i.e, summer in temperate area[illegible] and the rainy season in the tropics Th[illegible] transovarial transmission of rickettsiae i[illegible] these acarines facilitates maintenance of th[illegible] agent in nature, since the mite serves both a[illegible] vector and reservoir Various types of terrai[illegible] may serve as endemic areas, the commo[illegible] features of a focus are a suitable rodent population, adequate ground moisture favorable to the specific mite vectors, and the occurrence of *R. tsutsugamushi* in hosts of the area The[illegible]

volunteers and their prevention. Am J Hyg *51*, 229-241

Smadel, J E, and Jackson, E B, 1947, Chloromycetin, an antibiotic with chemotherapeutic activity in experimental rickettsial and viral infections, Science *106*, 418-419.

Smadel, J. E., Jackson, E B., Bennett, B L, and Rights, F. L., 1946a. A toxic substance associated with the Gilliam strain of *R orientalis*, Proc Soc Exper. Biol & Med *62*, 138-140

Smadel, J E, Ley, H L., Jr, Diercks, F H., and Cameron, J A P., 1952, Persistence of *Rickettsia tsutsugamushi* in tissues of patients recovered from scrub typhus, Am J Hyg *56*, 294-302

Smadel, J E, Ley, H L, Jr., Diercks, F H., and Traub, R., 1950b, Immunity in scrub typhus resistance to induced reinfection, Arch Path *50*, 847-861

Smadel, J E, Ley, H L., Jr., Diercks, F H, Traub, R., Tipton, V J, and Frick, L P, 1951, Immunization against scrub typhus I Combined living vaccine and chemoprophylaxis in volunteers Am. J Hyg *53*, 317-325

Smadel, J E., Rights, F L, and Jackson, E B, 1946b, Studies on scrub typhus II Preparation of formalinized vaccines from tissues of infected mice and rats Proc. Soc Exper Biol & Med *61*, 308-313

Smadel, J E, Traub, R, Frick, L P, Diercks, F H., and Bailey, C A, 1950c, Chloramphenicol (Chloromycetin) in the chemoprophylaxis of scrub typhus (tsutsugamushi disease) III Suppression of overt disease by prophylactic regimens of four-week duration Am J. Hyg *51*, 216-228

Smadel, J E, Woodward, T E., Ley, H. L., Jr, and Lewthwaite, R, 1949, Chloramphenicol (Chloromycetin) in the treatment of tsutsugamushi disease (scrub typhus), J Clin. Investig. *28*, 1196-1215

Snyder, J C., 1948, The treatment of the rickettsial diseases of man *in* The Rickettsial Diseases of Man, pp 169-177, Washington, D. C., Am Assoc. Advancement Sc.

Snyder, J C., Maier, J., and Anderson, C R., 1942, Report to the Division of Medical Sciences of the National Research Council, Washington, December 26

Topping, N H., 1945, Tsutsugamushi disease (scrub typhus) The effects of an immune rabbit serum in experimentally infected mice Pub Health Rep. *60*, 1215-1220

Topping, N H., and Shepard, C. C, 1946, The preparation of antigens from yolk sacs infected with rickettsiae, Pub Health Rep *61*, 701-707

Wissig, S L., Caro, L G., Jackson, E B, and Smadel, J E, 1956, Electron microscopic observations on intracellular rickettsiae, Am. J Path. *32*, 1117-1133

Zarafonetis, C J D., Ingraham, H S, and Berry, J F., 1946, Weil-Felix and typhus complement-fixation tests in relapsing fever, with special reference to *B proteus* OX-K agglutination, J Immunol *52*, 189-199

isolation of patients and quarantine measures are not indicated in this disease.

REFERENCES

Ahlm, C E, and Lipshutz, J, 1944, Tsutsugamushi fever in the Southwest Pacific Theater, J A M A *124*, 1095-1100

Audy, J R, and Harrison, J L, 1951, A review of investigations on mite typhus in Burma and Malaya, 1945-1950, Tr Roy Soc Trop Med & Hyg *44*, 371-404

Bailey, C. A, Diercks, F. H, and Proffitt, J E, 1948, Preparation of a serological antigen and a vaccine for experimental tsutsugamushi disease (scrub typhus) Research Project NM 005 002 (x-222), Report No 9, NMRU, National Naval Medical Center, Bethesda, Md

Bell, E J, Bennett, B L, and Whitman, L, 1946, Antigenic differences between strains of scrub typhus as demonstrated by cross-neutralization tests, Proc Soc Exper Biol. & Med *62*, 134-137

Bennett, B L, Smadel, J E, and Gauld, R L, 1949, Studies on scrub typhus (tsutsugamushi disease) IV Heterogeneity of strains of *R tsutsugamushi* as demonstrated by cross-neutralization tests J Immunol *62*, 453-461

Berge, T O, Gauld, R L, and Kitaoka, M, 1949, A field trial of a vaccine prepared from the Volner strain of *Rickettsia tsutsugamushi*, Am J Hyg *50*, 337-342

Blake, F G, Maxcy, K F, Sadusk, J F, Jr, Kohls, G M, and Bell, E J, 1945, Studies on tsutsugamushi disease (scrub typhus, mite-borne typhus)

1950, The influence of certain salts, amino acids, sugars and proteins on the stability of rickettsiae, J Bact *59*, 509-522

Bozeman, F M, Hopps, H E, Danauskas, J X, Jackson, E B, and Smadel, J E, 1956, Study on the growth of rickettsiae I A tissue culture system for quantitative estimations of *Rickettsia tsutsugamushi* J Immunol *76*, 475-488

Card, W I, and Walker, J M, 1947, Scrub-typhus vaccine field trial in South-east Asia, Lancet *1*, 481-483

Cox, H R, 1951, Some recent advances and current problems in the field of rickettsial diseases, Arch ges Virusforschung *4*, 518-533

Department of the Army Technical Bulletin TB

file in Department of Bacteriology, University of Maryland, College Park, Md

Doherty, R. L., 1956, A clinical study of scrub typhus in North Queensland, M J Australia *43*, 212-220

Fairley, Sir N H., 1951, Discussion of report of Audy, J R, and Harrison, J. L, 1951, Tr Roy Soc Trop Med & Hyg *44*, 399-400

Fox, J. P, 1949, The neutralization technique in tsutsugamushi disease (scrub typhus) and the antigenic differentiation of rickettsial strains, J Immunol *62*, 341-352

Fulton, F., and Joyner, L, 1945, Cultivation of *Rickettsia tsutsugamushi* in lungs of rodents preparation of a scrub-typhus vaccine, Lancet *2*, 729-733.

Jackson, E. B, 1951, Comparative efficacy of several antibiotics on experimental rickettsial infections in embryonated eggs, Antibiotics & Chemother *1*, 231-241.

Jackson, E B, Danauskas, J. X, Smadel, J E, Fuller, H S, Coale, M C, and Bozeman, F M, 1957, Occurrence of *Rickettsia tsutsugamushi* in Korean rodents and chiggers, Am J Hyg *66*, 309-320

Jackson, E B., and Smadel, J. E, 1951, Immunization against scrub typhus. II Preparation of lyophilized living vaccine Am J Hyg *53*, 326-331

Kawamura, R, 1926, Studies on tsutsugamushi disease (Japanese flood fever), M Bull College of Medicine, Univ of Cincinnati, *4*, 1-229.

Mackie, T T, Davis, G. E., Fuller, H S, Knapp, J A, Steinacker, M L, Stager, K E, Traub, R, Jellison, W. L, Millspaugh, D D, Austrian, R C, Bell, E. J, Kohls, G M, Wei, H, and Girsham, J A V, 1946, Observations on tsutsugamushi disease (scrub typhus) in Assam and Burma Preliminary report. Am J Hyg *43*, 195-218

Miesse, M, Diercks, F H, and Danauskas, J X, 1950, Strain differences among *Rickettsia tsutsugamushi* (abstract), Bact Proc, pp 90-91

Murray, E S, Zarafonetis, C J D, and Snyder, J C, 1945, Further report on effect of para-aminobenzoic acid in experimental tsutsugamushi disease (scrub typhus), Proc Soc Exper Biol & Med *60*, 80-84

Philip, C. B, 1948, Tsutsugamushi disease (scrub

313-317.

Sasa, M, 1954, Comparative epidemiology of tsutsugamushi disease in Japan (Studies on tsutsugamushi, part 76), Jap J Exper Med *24*, 335-361

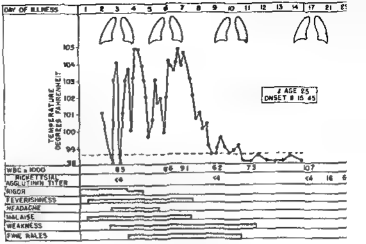
Smadel, J E, 1949, Evaluation of new drugs in the treatment of rickettsial diseases, Bull Pan American Sanitary Bureau *28*, 1-19

———, 1951, Present status of antibiotic therapy in viral and rickettsial disease, Bull New York Acad Med *27*, 221-231

———, 1956, Rickettsial diseases in Diagnostic Procedures for Virus and Rickettsial Diseases, ed 2, pp 513-556, New York, Am Pub Health Assoc

Smadel, J E, Bailey, C. A, and Diercks, F H, 1950a, Chloramphenicol (Chloromycetin) in the chemoprophylaxis of scrub typhus (tsutsugamushi disease). IV. Relapses of scrub typhus in treated

Fig 133 Chart of clinical findings in case of Q fever (*The Commission on Acute Respiratory Diseases, 1946, A laboratory outbreak of Q fever caused by the Balkan grippe strain of Rickettsia burneti,* Am J Hyg *44,* 133)



vealed the rickettsia to have a cosmopolitan distribution, its presence having been reported from 23 countries A more recent survey by Kaplan and Bertagna (1955) showed the rickettsia to be present in 51 countries on 5 continents Certain areas, e g , New Zealand, Sweden, Norway, Finland, Denmark, the Netherlands and Ireland, apparently are still free of Q fever

CLINICAL PICTURE

The incubation period is generally stated to range from 2 to 4 weeks and to average about 18 to 21 days (cf Lennette, 1956) However, studies on experimental Q fever in man showed that the incubation period in individuals infected by the respiratory route with the equivalent of one guinea pig inhalatory ID_{50} was 17 days (Tigertt and Benenson, 1956), infection with larger doses of rickettsiae produced progressive shortening of the incubation period to as little as 9 days

The disease usually begins abruptly with fever, chills or chilly sensations, headache, myalgia and anorexia During the first few days, the physical signs may be limited to fever, which ranges from 101° to 104° F Elevated temperatures of variable degree may persist from a few days to as long as 3 months (Clark et al , 1951c), the febrile period is less than a week in about one third of the patients and less than 2 weeks in about one half of the patients Usually a temperature of 102° F is exceeded at some time during the illness, but such temperatures are uncom after the 3rd week of illness The fev remittent in type, and the daily fluctua in temperature may be of considerable m tude whether or not antipyretics are ad istered Chilly sensations are common d the early stages of the disease Frank may occur, especially during the first days, and in severe infections may occu most daily for several weeks. Severe chill usually followed by profuse sweating, w may lead to subsequent dehydration H ache is a common symptom and is acteristically severe Disorientation and fusion occur in a small proportion of pati Stiffness of the neck or the back, s enough to suggest meningeal irritatio sometimes seen Anorexia is a common plaint, and this, as well as the nausea the vomiting which may be present, contr to the marked weight loss that may n Hepatic enlargement and tenderness are in a small proportion of patients, as is cal jaundice (Clark et al , 1951c)

Symptoms referable to the upper res tory tract are uncommon and usually sist of slight coryza or sore throat or a dry cough However, cough is not a prom symptom (Clark et al , 1951c, Tigertt Benenson, 1956), it is seldom present at but may appear from about the 5th da and is usually slight, dry and nonprodu

On examination of the chest, physical ings are usually minimal or absent, ev the presence of a pneumonic process,

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45

Q Fever

INTRODUCTION

Q fever is an acute systemic disease caused by a rickettsia (*Coxiella burnetii*, also known as *Rickettsia burneti*) and characterized by an abrupt onset with malaise, myalgia, severe headache, chilly sensations and high fever There is considerable variation in the severity and the duration of the disease An interstitial pneumonia frequently develops and, as is characteristic of other rickettsial diseases, rickettsemia occurs during the febrile phase However, Q fever, unlike other rickettsial diseases, is not accompanied by a cutaneous rash nor does it produce agglutinins against the so-called "X" strains of *Proteus vulgaris*

HISTORY

An outbreak of fever of unknown origin among abbatoir workers in Queensland, Australia, in 1935 was recognized by Derrick, 1937, to represent a new clinical entity He named the disease "Q fever", although it is sometimes referred to as "Queensland fever," this has no historical or geographic justifica-

named it *Rickettsia burneti*, after Burnet, whose studies had led to its classification as a rickettsia, Burnet and Freeman, 1937 At about the same time, Davis and Cox, 1938, isolated a filter-passing agent from ticks, *Dermacentor andersoni*, collected in the Nine Mile Creek area of Montana. The agent was subsequently classified as a rickettsia and, because of its filterability, was named *Rickettsia diaporica* by Cox, 1939. Comparative studies, Dyer, 1939; Burnet and Freeman, 1939, showed that *R. diaporica* was identical with *R. burneti* (now *Coxiella burnetii*) and thus suggested that Q fever was present in the United States Serologic surveys (Cox, 1940) showed that natural infection of man occurred in the Western United States, but the occurrence of clinically recognizable, naturally acquired disease was thought for some years to be restricted to Australia However, during the winter and the spring of 1944-1945, several outbreaks of pneumonitis, later proved to be Q fever, occurred among Allied troops in the Eastern Mediterranean area, more than 1000 cases were recorded (Robbins et al, 1946a, Commission on Acute Respiratory Diseases, 1946a) During the preceding winter, outbreaks of a disease referred to as "Balkan grippe" had occurred among "Axis" troops, an agent recovered from the blood of one of the patients by Caminopétros was later identified as *R burneti* (Commission on Acute Respiratory Diseases, 1946b) These represent the first naturally occurring outbreaks of Q fever recognized outside Australia. In 1946, outbreaks occurred in Amarillo, Texas (Cox et al, 1947) and Chicago meat-packing houses (Shepard, 1947), and in 1947 Q fever was found to be endemic in California (Shepard and Huebner, 1948). Numerous reports on Q fever appeared during the succeeding few years, and a literature survey by Berge and Lennette (1953a) re-

are seen more commonly in Q fever The lesions in atypical pneumonia consist of patchy and mottled infiltrations, whereas the most frequent lesions in Q fever are homogeneous consolidations Roentgenographically, Q fever closely resembles pneumococcal pneumonia (Jacobson et al, 1949); this is of interest in view of observations (Whittick, 1950) that the gross appearance of the consolidated lung at postmortem simulates that seen in pneumococcal lobar pneumonia but histologically is quite different.

Complications are not common As the temperature approaches normal, the patient's appetite returns, and there are no sequelae; a feeling of weakness or fatigue may be present for some weeks and, occasionally, months Severely ill patients may lose considerable weight (15 to 20 lbs) during the course of the disease, but such losses are remedied rapidly. Clark et al (1951c) emphasize that the disease may take a protracted course, and that fever lasting a month or more is seen not infrequently in persons over 40 years of age Evidence of hepatic involvement, including clinical icterus, was observed by Clark et al (1951c) in nearly one third of the individuals with a severe and protracted illness Thrombophlebitis, pleurisy, pleural effusions and leg pains have been reported as complications (Huebner et al, 1949a), but thrombophlebitis and leg pains resembling those of intermittent claudication have been the most commonly encountered sequelae (Huebner et al, 1949a, Clark et al, 1951c) Relapses may occur following apparent recovery (Lennette et al, 1948, Clark and Lennette, 1952, Huebner et al, 1949a)

The routine clinical laboratory tests are of little assistance diagnostically The red cell count and the hemoglobin level remain normal The total white cell and differential counts tend to remain within normal limits, but wide variations may occur and do not exclude Q fever from diagnostic consideration (Clark et al, 1951c) The only abnormal finding consistently encountered is an elevated sedimentation rate, the rate increases promptly with onset of clinical illness and apparently is higher in individuals with roentgenographic evidence of pulmonary involvement than in those without it (Tigertt and Benenson, 1956) A mild albuminuria is present during the febrile period The cold agglutination and the streptococcus MG agglutination tests are negative. Specific laboratory diagnosis of Q fever is discussed later in this chapter.

Studies on experimentally induced infections in man have yielded important and interesting information on the disease process Blanc et al. (1948) produced infection in man by intradermal, intramuscular or respiratory inoculation Intradermal inoculation produced a small local cutaneous lesion and a mild fever of brief duration, intramuscular inoculation produced considerable local swelling and a marked fever which persisted over several days; infections produced by these routes were, on the whole, inapparent However, inhalation of an infective aerosol produced a pneumonitis and typical symptoms of Q fever. In the experiments of Fonseca et al. (1949) inoculation by the respiratory route produced primarily inapparent infections Intradermal inoculation elicited a local cutaneous reaction and sometimes slight general symptoms Subcutaneous inoculation produced infections clinically typical of Q fever It is of interest (cf Epidemiology) that ingestion of infected material neither produced symptoms of infection nor elicited an antibody response Tigertt and Benenson (1956) showed that inhalation of infectious aerosols produces typical Q fever, that a pneumonitis is not an invariable concomitant of the disease process is indicated by their finding that only one half of the patients had evidence of pulmonary involvement on repeated roentgenographic examination It is of interest that the development of pneumonic lesions bore no relationship to the size of the infecting dose

PATHOLOGIC PICTURE

Q fever is rarely fatal The death rate is stated to be about 1 per cent or less among Caucasians and somewhat higher among the indigenous peoples of equatorial Africa (Babudieri, 1953a) Only a half dozen or so fatalities have been recorded in the literature (cf Whittick, 1950), and postmortem examinations were performed on some of these cases (cf Lillie, et al, 1941; Perrin, 1949, Whittick, 1950) In most of these cases, death was due to a diffuse pneumonia of lobar or greater extent The gross appearance of the consolidated lung closely resembles that

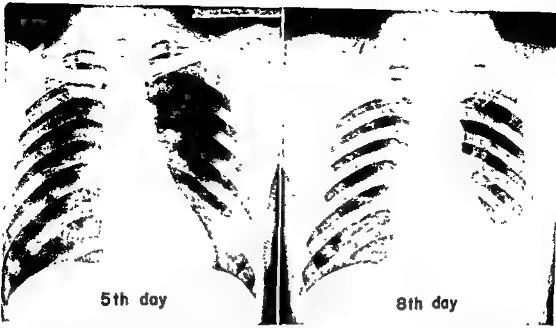

FIG 134 Roentgenogram of chest of patient with Q fever, whose chart is shown in Fig. 133. (The Commission on Acute Respiratory Diseases, 1946, A laboratory outbreak of Q fever caused by the Balkan grippe strain of *Rickettsia burneti*, Am J. Hyg *44*, 133)

elicited, they are over the area of involved lung. Careful examination may detect a few crepitant rales, a slight diminution of breath sounds, increased vocal fremitus or impaired resonance Figure 133 summates the salient data from the hospital record of an individual with a laboratory infection (Commission on Acute Respiratory Diseases, 1946c); roentgenograms of the chest of this patient are shown in Figure 134

Q fever is often regarded as a respiratory disease, because many patients develop a pneumonitis similar to that found in viral pneumonias and atypical pneumonias and demonstrable mainly by roentgenographic examination Comprehensive studies on patients involved in several laboratory outbreaks of Q fever (Hornibrook and Nelson, 1940, Spicknall et al , 1947; Robbins and Rustigian, 1946; Commission on Acute Respiratory Diseases, 1946c) revealed roentgenographic evidence of pulmonary involvement in more than 60 per cent of the individuals examined Roentgenograms have been used to detect inapparent and mild infections (Feinstein et al., 1946) but, unfortunately, pneumonitis has been so widely stressed as an integral part of the clinical picture of Q fever that its presence is sometimes considered as virtually a requisite for the establishment of a diagnosis of Q fever Q fever is a systemic disease and should not be regarded as solely a pneumonitis. In the writer's experience (Lennette and Meiklejohn, 1948; Clark et al , 1951c), roentgenographic evidence of pulmonary involvement was seen in only one third of the patients so studied. In their studies of experimental infections in man, Tigertt and Benenson (1956) likewise obtained roentgenographic evidence of pneumonia in only one half of their cases These authors also found that recognizable lesions may be present at the time of onset of fever and that the occurrence of pneumonitis was unrelated to the size of the infecting dose.

Roentgenographic changes seen in the lung are generally considered to resemble those of the viral pneumonias and the primary atypical pneumonias. However, Feinstein et al (1946) and Jacobson et al. (1949) consider Q fever to present certain distinctive roentgenographic features of its own. According to the latter workers, the hilar and vascular engorgement seen in many atypical pneumonias is absent in Q fever. Although encountered in both diseases, segmental or lobar infiltrations

in the inoculum, but the absence of such a nodule does not necessarily mean the converse. The microscopic changes seen in infected guinea pigs consist of small focal granulomata scattered throughout practically all the organs and tissues. The lesions are characterized by a vascular endotheliosis and perivascular collections of lymphocytes, less often of monocytes and fibroblasts (Lillie, 1942)

For those experimental and investigative purposes in which it is not necessary to measure the febrile response of the test animal, the hamster possesses certain definite advantages over the guinea pig Thus, the hamster is a less expensive test animal, requires less care and is much more resistant than the guinea pig to intercurrent infections In the writer's laboratory, the hamster is used almost exclusively for Q fever studies, the occurrence of infection in the inoculated test animal is determined by examining the serum for the presence of antibodies at 4 to 6 weeks after inoculation

C. burnetii is readily propagated in tissue cultures and in embryonated eggs. Embryonated eggs are inoculated into the yolk sac at 5 or 6 days of age Relatively concentrated inocula produce death of the embryo within 4 or 5 days' incubation at 35° C, and the lining cells of the sac yield a rich harvest of rickettsiae The yolk-sac tissues obtained from dead or moribund embryos provide the basic starting material for the preparation of stock rickettsial suspensions diagnostic antigens, vaccines, etc

ETIOLOGY

C burnetii is an obligate, intracellular, parasitic micro-organism It is small and pleomorphic, occurring as a diplobacillus 0 25 $\mu \times 1.5\ \mu$, a bipolar rod 0 25 $\mu \times 1.0\ \mu$ or as a lanceolate rod 0 25 $\mu \times 0.5\ \mu$ The rickettsia stains poorly by Gram's method (gram negative) but takes a purple color when stained by Giemsa's method and a red color when stained by Macchiavello's method The organisms occur as clumps and masses, usually within the cytoplasm of endothelial and serosal cells as, for example, those lining the peritoneum and also in the peritoneal exudate of mice and other animals In some preparations, the organisms may be mistaken for gram-negative bacteria because of their large size, but the same preparations will also reveal the presence of small particles with the same staining properties as the rodlike bodies The presence of very small infective particles in a homogenate of tissue infected with *C. burnetii* is revealed not only by microscopy but also by the fact that the infective property is present in filtrates of homogenates passed through collodion membranes with an average pore diameter of 400 mμ (Bengtson, 1941a) As mentioned earlier, this unusual property of filterability led to the name *Rickettsia diaporica* for this organism, Cox, 1939

Electronmicrographs show *C burnetii* to possess a limiting membrane that encloses protoplasmic material in which are interspersed granules of a denser material, comparison with other rickettsiae reveals no practical bases for the morphologic differentiation of one rickettsial species from another

C burnetii possesses properties not found in other members of the family *Rickettsiaceae*, thus, it is filterable, highly resistant to physical and chemical agents, does not elicit the so-called "X" agglutinins responsible for the Weil-Felix reaction and does not produce the cutaneous rash associated with other rickettsial diseases of man Because of these differences, Philip (1948) proposed that the Q fever agent be removed from the genus *Rickettsia* and serve as the prototype for a new genus to be known as *Coxiella* after H R Cox; the genotype was to remain the same The new name, *Coxiella burnetii* (Derrick) is listed in Bergey's *Manual of Determinative Bacteriology*, and this usage is followed here Establishment of the genus *Coxiella* has not been universally accepted, and the designation *Rickettsia burnetii* is still widely employed, especially outside the United States

C burnetii is markedly resistant to desiccation This property is important in the transmission of the disease (cf. Epidemiology), since desiccated tick tissues and tick feces, and the desiccated excreta and secretions of infected livestock contain fully viable organisms The organisms remain viable for days in water or in milk, can withstand drying at ambient room temperatures and can be preserved over long periods of time by storage in the frozen state at −20° or −70° C, and

seen in pneumococcal lobar pneumonia However, the histologic picture is quite different from that of pneumococcal pneumonia and closely resembles that seen in psittacosis and other viral pneumonias.

Microscopically, the histologic picture seen depends upon the stage in the pneumonic process at which the tissue is examined In areas of red hepatization, consolidation is uneven, and the distribution of affected alveoli is patchy and irregular The alveoli are filled with a red-staining coagulum containing a small number of neutrophilic polymorphonuclear cells and round cells in about equal proportions The round cells are chiefly mononuclear cells, although lymphocytes and plasma cells are present Red cells are sparse and seen in only an occasional alveolus An exudate similar to that in the alveoli is present in the bronchioles, and there is edema of the interlobular connective tissue

In areas of gray hepatization, the interalveolar septa are considerably thickened by infiltrations of lymphocytes, plasma cells, large mononuclear cells and neutrophils, fibroblasts, focal necrosis of some of the alveolar

nuclear cells, although numerous red cells and some neutrophils are present. The bronchioles contain a similar cellular exudate and generally show necrosis and desquamation of epithelium The subpleural and interlobular connective tissues show edema and round-cell infiltration

Whittick (1950) has demonstrated the presence of rickettsiae in large numbers in lesions in the lung, the spleen, the testes, the brain and the kidneys The organisms were found both intracellularly and free outside the cells In the lung, the spleen and the testes, the organisms were found within macrophages, and in the brain they were found within neuroglial cells In the kidneys, rickettsiae were found within the tubular epithelium, free in the lumen and also within cells of the intertubular connective tissue

EXPERIMENTAL INFECTION; HOST RANGE

The host range of *C. burnetii* is very broad, including numerous species of ticks, several insects, many species of wild and domesticated animals, and a number of birds (Weyer, 1953, Stoker and Marmion, 1955; Babudieri, 1953a; Syrůček and Raška, 1956, Raška and Syrůček, 1956). Evidence that some of these species are susceptible to natural infection has been obtained by isolation of the rickettsia or by detection of specific antibody in the blood. In other instances, susceptibility to infection has been determined (or confirmed) experimentally.

Mice, hamsters, guinea pigs and embryonated eggs have been employed in most of the investigative work on Q fever Infected mice rarely succumb to the disease, therefore, the existence of infection is generally determined by microscopic examination of the peritoneal exudate or of splenic imprints for the presence of rickettsiae Despite the lack of overt evidence of infection, the rickettsia multiplies freely, since mouse spleen tested in other species (e g , the guinea pig) gives infectivity titers of 10^{-8} or more.

Guinea pigs inoculated with *C burnetii* develop a fever, the interval between inoculation and the appearance of a febrile response depending to some extent upon the number of rickettsiae injected. Most strains of *C. burnetii*, and especially field strains at low passage levels, are not lethal for guinea pigs even when highly concentrated inocula are used However, certain strains, such as the Henzerling strain from Italy and the AD strain from California (Huebner et al , 1948) are lethal when inoculated in high concentrations, lower concentrations of rickettsiae result in infection The difference between the ID_{50} and the LD_{50} titer of such strains may be as great as 8 or 9 logarithmic units The organisms persist in the tissues of convalescent animals for many months and may be excreted in the urine over similar long periods of time (Parker and Steinhaus, 1943)

The gross pathologic changes in guinea pigs are generally limited to enlargement of the spleen, which may be from 2 to 4 times its normal size Intraperitoneal inoculation is characterized by the appearance of an exudate which covers the viscera and contains rickettsiae. Subcutaneous inoculation may elicit a firm nodule at the injection site, but such a lesion, in the author's experience, is more often called forth by laboratory passage strains than by field materials Thus, the development of such a nodule may be taken as a clue to the possible presence of rickettsiae

It thus appears that the strain to be used for the preparation of complement-fixing antigen should be chosen on the basis of the geographic area or the animal species to be tested, or both.

Apparently, the factors responsible for such differences in complement-fixing reactivity between strains are not operative in agglutination tests (Robbins et al, 1946; Stoker et al., 1955b) It seems likely that this may prove to be an important point in any future considerations of agglutination technics for diagnostic work

Animals recovered from infection with one strain develop resistance to reinfection with homologous or heterologous strains (Dyer et al, 1940, Topping et al, 1946) However, resistance is relative and may be overwhelmed by the use of potent challenge inocula; depending upon the balance between degree of resistance and size of challenge dose, reinfection may result in only a febrile response or may terminate fatally Animals recovered from infection with *C burnetii* do not acquire resistance to infection with other rickettsiae.

Some protection against infection with *C burnetii* can be conferred by administration of noninfectious vaccines prepared from animal or egg tissues rich in *C. burnetii* (Smadel et al, 1948) However, vaccination does not produce solid immunity, and the vaccinated animals are able to resist completely only a few minimal infectious doses of the homologous or heterologous strain; inoculation of larger doses results primarily in a febrile response, seldom death

DIAGNOSIS

The diagnosis of Q fever in man is established by isolation of the causal rickettsia or by demonstrating that specific antibodies against *C burnetii* have appeared, or increased in titer, in the patient's serum during the course of the illness The history and the clinical findings are generally insufficient to permit a diagnosis, but the disease should be suspected in any patient whose epidemiologic background reveals direct or indirect exposure to livestock by virtue of occupation or residence During the first few days after onset, the illness may resemble the early phases of many acute infectious diseases such as influenza, brucellosis, typhoid or paratyphoid fevers, infectious hepatitis, leptospirosis, meningitis, sandfly fever, dengue fever, malaria, other rickettsial diseases, etc Differential diagnosis in those patients who develop a pneumonitis necessitates consideration of bacterial pneumonia, psittacotic and other viral pneumonias, primary atypical pneumonia and, in certain geographic areas, coccidioidomycosis

C burnetii can be isolated from the sputum, the urine or the blood (sometimes the spinal fluid) of a patient, or from tissues obtained postmortem Since rickettsemia is present during the febrile phase of the illness, isolation is most simply accomplished by the use of blood. Guinea pigs, hamsters and embryonated eggs are the laboratory host species most commonly employed. Identification of the agent is done as follows. Guinea pigs are inoculated intraperitoneally with clinical material from the patient Animals which develop a fever are killed, the spleens are removed and passaged intraperitoneally into additional animals Several passages may be required, as rickettsiae may not be detectable in splenic smears made during the early passages When splenic imprints stained by Giemsa's or Macchiavello's method reveal the presence of rickettsiae, cross-immunity tests are performed in animals immunized to *C burnetii* and other agents A much simpler animal test is performed as follows. a group of guinea pigs is inoculated intraperitoneally with material from the patient, bled from 4 to 6 weeks later, and the individual sera are examined for the presence of specific complement-fixing or agglutinating antibodies to *C burnetii*. This method is used in the author's laboratory, the hamster being used instead of the guinea pig. Because of the very real infection hazard, not only to those actually working with the rickettsia but also to [illegible]

working area

Serologic methods are simpler and safer than isolation of the causal agent and constitute the procedures of choice in diagnostic laboratories Of the several methods for measuring antibody, the most widely employed are the agglutination test and the direct complement-fixation test The complement-fixa-

after lyophilization. According to Babudieri (1953a), it can withstand temperatures of 50° C, for at least 30 minutes and often temperatures of 60° and 70° C. for shorter periods; dried and exposed in a thin layer to irradiation by an ultraviolet lamp, it will survive for 30 minutes (Babudieri, 1953a). *C. burnetii* can withstand the action of 0.5 per cent formaldehyde for 24 hours and 0.2 per cent formaldehyde or 0.4 per cent phenol for several days.

Specific antigens for complement-fixation and agglutination tests are derived from infected yolk sacs which are rich in rickettsial content and the material of choice for this purpose. In passing, it is of interest that the first satisfactory rickettsial complement-fixing antigen prepared from yolk sac material was that for Q fever (Bengtson, 1941b).

All strains of *C. burnetii* are not equally suitable for making complement-fixing antigens. Guinea pigs infected with any strain apparently develop 2 complement-fixing antibodies. One antibody appears within 3 weeks after infection, and the other appears several weeks later, by the 9th or 10th week, the titer of the second antibody approaches the levels reached earlier by the first antibody. While most strains of *C. burnetii* contain complement-fixing antigens that react with the second antibody, certain strains appear to lack, or be relatively deficient in, the antigenic component which combines with the first antibody to appear (Robbins et al., 1946).

The Henzerling strain, isolated in Italy, was originally chosen for diagnostic work, since it contains antigens that react with both types of antibody (Robbins et al., 1946). Berge and Lennette (1953b) found that strains obtained from a variety of sources differed widely in their sensitivity as complement-fixing antigens when tested with early convalescent sera but that, in many instances, they were almost equally good as antigens when tested with sera taken some months after recovery. They also found that the so-called classic strains, viz., Dyer, Henzerling and Nine Mile, reacted equally well with both very early and very late convalescent sera. The differences in sensitivity or reactivity of an antigen with two types of antibody appears due, in some part, to the extent to which the rickettsial strain is adapted to growth in yolk-sac tissue. The studies of Stoker et al. (cf Stoker and Marmion, 1955) have shown that strains with only a few yolk-sac passages fail to react in complement-fixation tests with homologous or heterologous Q fever antisera, although the antisera react well with antigens of similar rickettsial concentration prepared from classic strains such as the Henzerling or the Nine Mile. However, after a variable number of yolk-sac passages, the new strains yield antigens which, although no richer in rickettsial content than antigens prepared from early passage material, resemble the classic strain antigens. This adaptive process has been named "phase variation" by Stoker. Antigens prepared from rickettsiae in the early stage of egg adaptation, which is called "phase 1," show no reactivity with early convalescent sera from naturally or artificially infected animals. After adaptation to the egg ("phase 2"), the same rickettsial strain yields antigens that react well with both early and late convalescent sera. (When strains in phase 2 are passaged in animals, they revert to phase 1 as shown by subcultivation of the animal passage material in the egg; several yolk-sac passages are required to reconvert the strain to phase 2.)

Factors other than phase variation are concerned in the reactivity or the sensitivity of a complement-fixing antigen. Thus, the Henzerling and the Nine Mile strains are widely used as complement-fixing antigens for diagnostic purposes since they react equally well with early and with late convalescent sera, react very well with antisera prepared against heterologous strains in guinea pigs and give good fixation with the sera of patients from areas other than the geographic point of origin of these strains. However, recent evidence indicates that even these 2 classic strains cannot be regarded as interchangeable. Thus, in at least some parts of England, the Nine Mile antigen has been found to be more sensitive than the Henzerling antigen for the detection of complement-fixing antibodies in man (Stoker et al., 1955b). The Nine Mile strain was more sensitive for the detection of antibodies in the sera of sheep from Wales, whereas the Henzerling antigen was markedly more sensitive than the Nine Mile antigen for the detection of antibodies in sheep from Kent.

to a small quantity of *C. burnetii* were not protected; 4 of 5 volunteers developed clinical symptoms after an incubation period that was prolonged for 8 or 10 days beyond that of the controls. The patients responded promptly to therapeutic administration of additional oxytetracycline, and no relapses were encountered.

The suggested antibiotic therapy for adult patients with Q fever is 2 to 3 Gm. daily of oxytetracycline, chloramphenicol or chlortetracycline administered orally; a regimen similar to the one above may be followed. Since these antibiotics are rickettsiostatic rather than rickettsiocidal, their administration should be continued for several days after the temperature has returned to normal in order to prevent relapses. If relapses occur, therapy should be reinstituted and continued again for several days after the temperature reaches normal levels.

Penicillin, streptomycin and the sulfonamides possess no therapeutic value in Q fever.

EPIDEMIOLOGY

The wide distribution of *C. burnetii* in nature—in ticks, human body lice, small wild animals, cattle, sheep, goats, birds and man—suggests that the epidemiology of Q fever in different parts of the world may vary according to the geographic and environmental factors present.

The fact that at least 22 species of tick, representing 6 genera of *Ixodid* and 2 genera of *Argasid* ticks, have been found infected (cf. Weyer, 1953; Stoker and Marmion, 1955) indicates that this arthropod plays an important role in the maintenance of the infection in nature. These ticks have come primarily from animals known to be susceptible to infection with *C. burnetii*, but it cannot be assumed that all are equally important as vectors of the disease. Thus, laboratory studies have shown that while all species that have been tested can be infected by feeding on infected animals, not all are capable of transmitting the infection. Also, although stage to stage ...

... tick generations depends upon reinfection of the arthropod host from a vertebrate host. The situation with respect to the epidemiologic role of ticks may be summarized by pointing out that while many species of ticks in various parts of the world are susceptible to infection with *C. burnetii*, and many are known to transmit the organism under laboratory conditions, little is known of the absolute or relative efficiency with which this arthropod transmits under natural conditions. As will become evident further below, the tick has little importance in the transmission of Q fever to man, and similarly it plays, in some parts of the world at least, a very minor role in the transmission of the rickettsia to domesticated livestock. Since *C. burnetii* has been found in many species of ticks, which at some stage of their life cycle feed on small wild animals, and since many of these animal hosts have been found susceptible to experimental infection, it is believed that an animal-tick-animal cycle is the basic mechanism responsible for maintenance and perpetuation of the rickettsia in nature.

Derrick's early studies incriminated cattle as a possible source of infection for man. Serologic surveys revealed that cattle were infected, and subsequently a deliberate search for an arthropod vector of the rickettsia was undertaken. The work of Derrick and his coworkers showed (Derrick, 1944, 1953) that in Australia Q fever is probably an enzootic infection of a number of small wild animals but especially of the bandicoot. About 30 per cent of the bandicoots on Moreton Island were found to possess agglutinins to *C. burnetii*, and the rickettsia was isolated both from bandicoots and from a tick, *Haemaphysalis humerosa*, with which they were heavily infested. *Ixodes holocyclus* also attacks the bandicoot, and both this species and *Haemaphysalis humerosa* ...

... tion through the mediation of a tick attacking both the bandicoot and cattle. *H. humerosa* rarely feeds on cattle and thus appeared unlikely to carry the infection from one animal species to the other. Derrick (1944) believed that infection in the bandicoot population might spill over into cattle via *Ixodes holocyclus*. This appears to be a distinct possibility since naturally infected *I. holocyclus* have been found recently on cows (Carley and Pope, 1953). Cattle ticks (*Boophilus annulatus microplus* and *Haemaphy-*

tion test has been generally preferred to the agglutination test since it is cheaper, more easy to perform and simpler to read The Henzerling and the Nine Mile strains are generally regarded as the antigens of choice, but the admonition given above (cf Etiology) to the effect that the choice of antigen may have to be based on the geographic area from which the test sera come and on the animal species from which they are obtained should be kept in mind.

The agglutination test has had a rather limited acceptance in diagnostic work but may be utilized much more widely in the future when the comparative disadvantages mentioned above are overcome Recently, micromethods using slides (Babudieri and Secchi, 1952, Babudieri, 1953b) or capillary tubes (Luoto, 1956) have been introduced Both technics are simple to perform and give reproducible results The slide agglutination method has been found, both in Babudieri's laboratory and in the author's laboratory, to be more sensitive than the complement-fixation method, perhaps this greater sensitivity may be referable to the absence or the inoperativeness of those elements which so markedly affect the sensitivity of different strains of *C burnetii* as complement-fixing antigens Precise comparative studies on the complement-fixation and the various agglutination technics are needed to evaluate the merits of the various procedures

Agglutinating antibodies appear early after the onset of illness and are demonstrable in about 50 per cent of the patients during the 1st week, the proportion of patients with agglutinins rises to 92 per cent during the 2nd week and reaches 100 per cent by the 4th week (Lennette et al, 1952b) In contrast, complement-fixing antibody appears somewhat later than does the agglutinin, it is encountered only occasionally during the 1st week of the illness, rises to 65 per cent during the 2nd week and reaches about 90 per cent at 1 month after onset Both agglutinating and complement-fixing antibodies may persist in high titer over many months

The serologic reactions obtained with Q fever antigens are highly specific, and no cross-reactions occur with any of the other rickettsial agents nor with a variety of viral agents that have been tested As is true in other viral and rickettsial infections, paired or multiple blood specimens taken during the acute and recovery, or convalescent, phases of the illness should be examined for the appearance, or rise in titer, of specific antibodies to *C. burnetii* during the course of the illness A serologic diagnosis based on these criteria rests on a much more solid footing than does one based on a so-called "diagnostic titer" determined by examination of a single blood specimen.

TREATMENT

Treatment of infected eggs with chlortetracycline (Wong and Cox, 1948), chloramphenicol (Smadel et al, 1949) and oxytetracycline (Smadel et al, 1950) has shown clearly that *C. burnetii* is highly vulnerable to the action of these antibiotics However, the efficacy of these antibiotics in the treatment of human Q fever has been difficult to evaluate because of the considerable variations in the severity and the duration of the untreated disease Several studies indicate that chlortetracycline is useful in the treatment of Q fever (Lennette et al, 1948; Clark et al, 1951b; Clark and Lennette, 1952)

Chloramphenicol (Harman, 1949; Zarafonetis and Bates, 1950) and oxytetracycline (Bickel and Plattner, 1951, Tigertt and Benenson, 1956) also have a specific therapeutic action This is clearly shown for oxytetracycline in the human volunteer studies of Tigertt and Benenson (1956) Oxytetracycline therapy was initiated within 24 hours after the onset of persistent fever in 29 volunteers who were infected by the inhalatory route The drug was usually given orally, the regimen consisting of a 3-Gm priming dose followed by 0 75 Gm. every 6 hours for a total average dose of 20 Gm over a 5- to 6-day period Cessation of symptoms occurred within 24 to 48 hours after therapy was initiated. No relapses occurred in any of the 29 clinical cases studied Prophylactic administration of oxytetracycline was studied in comparable groups of volunteers using a similar regimen of 20 Gm of oxytetracycline over a 6-day period When oxytetracycline prophylaxis was initiated late in the incubation period, clinical illness was prevented in every instance However, men placed on the same prophylactic schedule within 24 hours after exposure

(Clark et al, 1951c; 1951f). Infection was found to be widely prevalent in sheep, and although an appreciable proportion of infected animals excreted the rickettsia in the milk, such excretion was essentially confined to a very short period postpartum and would not account for the occurrence of the large number of human cases observed. Consequently, other sources of rickettsiae were searched for, and it was found that naturally infected sheep excrete *C. burnetii* not only in the milk (Lennette et al., 1949) but also in the placenta (Welsh et al., 1951, Stoker et al, 1955a), birth fluids (Abinanti et al, 1953a) and feces (Winn et al, 1953) at, or for a short time after, parturition. The placenta especially may be very heavily infected and contain as high as 10^9 hamster infective doses per Gm Luoto and Huebner (1950) also found that the organism is excreted in the placenta of the infected cow, and that the placental tissues may be very rich in rickettsial content. Experimental studies by Lennette et al (1952c), and Abinanti et al (1953b) showed that sheep can be infected by the intravenous and, much more importantly, by the respiratory route After intravenous inoculation the rickettsiae were found to localize in various organs in which they could be detected up to the end of the 6-week observation period, except for several isolated instances, no shedding of the organisms occurred via mammary gland secretions, the urine, the feces or by way of the oral or nasal secretions. Similarly, after intratracheal inoculation no remarkable excretion of rickettsiae by any of these routes was encountered, however, several of the inoculated animals lambed some months after inoculation, and the rickettsiae immediately appeared in the oral and nasal secretions and in the urine and the feces of the parturient animals Further studies show that during parturition, infective aerosols are generated and lead to demonstrable infection of the aerial environment (Welsh et al., 1957) The Northern California workers were also able to detect the presence of rickettsiae in the air of premises harboring infected cows, goats and sheep (De Lay et al, 1950, Lennette and Welsh, 1951). The results of the California studies indicate that in that area arthropods play no role in the transmission of infection among livestock or

in man may occur from primary aerosols generated during parturition of the animal. Infection may also be acquired from secondary aerosols, i e , contaminated dust, hides, fleece, etc., since desiccation of infected birth fluids and placentas as well as of other secretions or excretions gives rise to an environment in which aerosols of infective dust can be created. It is of interest that in Northern California the peak incidence of human cases occurs at the time of the lambing season, whereas in Southern California human cases

from the reproductive tract of one animal to the respiratory tract of another; infec-

tion of infected milk or colostrum, but the available evidence suggests that the respiratory route is the more important.

Man may acquire his infection by direct contact with livestock, i e , through direct exposure to infectious aerosols generated by the act of parturition or by direct exposure to

However a large proportion of cases of Q fever occurs among individuals with no history of direct or even indirect contact with livestock; such cryptic infections may occur as sporadic cases or in the form of outbreaks (cf Clark et al , 1951f) The Northern California group (Clark et al , *1951e, 1951f*) have stressed the importance of infected "micro-environments" as a source of infection in such cryptic cases. Thus, in one outbreak infections occurred predominantly among individuals with indoor pursuits, e g, attorneys, court clerks, a judge,

occupationally engaged, and that infection probably was brought to them in the form of mobile micro-environments

importance of such micro-environments is in-

salis bispinosa) may in turn acquire the rickettsia by feeding on their infected hosts. Whether cattle ticks can spread the infection through a herd is uncertain, especially in the case of *B microplus* which spends its entire life on one animal. The tissues and especially the feces of such infected cattle ticks may contaminate the hides of their hosts and thereby give rise to a potential infection hazard of man

A wild animal-tick-domestic animal association similar to that just described appears to exist in other parts of the world, e g., Morocco and Spain In Morocco the gerbil (merion rat or desert rat) *Meriones shawi* is heavily parasitized by ticks of the genus *Hyalomma*, and infected ticks of *Hyalomma spp.* have also been found on sheep, goats and cattle (Blanc and Bruneau, 1949). In Spain, *Hyalomma marginatum*, *Rhipicephalus bursa* and *R. sanguineus* have been found on livestock, and both cattle and small mammals (dormice and mountain rabbits) have been found to be infected (Pérez Gallardo et al , 1949; 1952). It thus seems possible that in certain areas, at least, the tick may serve to carry the infection outside its basic cycle in small animals to the domestic animals of man.

With rare exceptions, patients with Q fever give no history of tick-bite Early studies indicated that Q fever was an occupational disease apparently acquired by direct contact with infected meat or by inhalation of infected dust from cattle hides contaminated with infected tick tissues or tick feces. Inasmuch as *C. burnetii* remains viable in ticks over periods of many months, and since the tissues and the excreta of infected ticks contain tremendous concentrations of rickettsiae, such materials may well constitute a source of infection under some circumstances, for example, the outbreak of Q fever among stock handlers in Amarillo, Texas. Indeed, infection by the respiratory route affords the only possible, and logical, explanation of a number of outbreaks in military and civilian populations and especially of outbreaks which occurred in laboratories (Robbins et al., 1946a, Commission on Acute Respiratory Diseases, 1946c; Topping et al., 1947; Wegmann, 1948; Huebner, 1947; Kikuth and Bock, 1949; Hornibrook and Nelson, 1940).

The localized nature of some outbreaks and the tendency to affect primarily individuals coming within certain areas (e g , Army billets, houses, farms, etc.) pointed to Q fever as a "place disease." The explosive nature of other outbreaks pointed to a common source of infection for man. This was true in Italy, for example (Robbins et al., 1946a) where epidemiologic evidence ruled out person-to-person transfer, and it was not possible to incriminate food or water. The Italian outbreaks were found to be associated with animal life—birds, rodents and domestic livestock—and it was considered that these or oth[illegible]

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houses used as billets and on hay and straw used [illegible] bedding material.

Much of our information on the natural sources that give rise to such outbreaks comes from field studies in the United States. The discovery of human cases of Q fever in Southern California in 1947 led to intensive epi-

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Los Angeles area had serologic evidence of infection, that nearly one half of the immigrant cattle coming from nonendemic areas became infected within 6 months after arrival, and that *C. burnetii* was excreted in the milk of a large proportion of the dairy cattle Since the raw milk of more than half of the dairies tested in the area contained readily demonstrable *C. burnetii* (Huebner et al , 1949a, Huebner et al , 1948) and since complement-fixing antibodies to *C burnetii* were encountered more frequently among drinkers of raw milk than among users of pasteurized milk (Bell et al , 1950), contaminated milk appeared to be a possible source of human infection However, the ingestion of raw milk as a cause of human Q fever would not explain the occurrence of the disease in a considerable proportion of individuals who did not use raw milk (Bell et al., 1950, Lennette and Clark, 1951). In addition, the studies of Fonseca et al. (1949) showed that man is

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finding that in Northern California human cases of the disease gave a history of direct or indirect contact with sheep and goats rather than with cattle, and the milk of these former species was not utilized as a food product (Lennette et al , 1949; Lennette and Clark, 1951).

Studies in Northern California pointed to sheep as a source of infection and to the respiratory tract as a portal of entry in man

ranted However, sterilization of sputum and excreta is recommended, and precautions should be taken in the handling, or the post-mortem examination, of individuals dead of Q fever. These recommendations are based on such episodes as that described by Siegert et al (1950) in which a hospitalized patient infected 38 other individuals, including patients and members of the staff Marmion and Stoker (1950) report several cases which occurred in connection with an autopsy, and Babudieri (1953a) mentions an episode in which 16 of the 17 individuals present at the postmortem on an individual who died of Q fever were infected

Care must be exercised in other respects also, and material which can even remotely be considered as contaminated should be sterilized Thus, an outbreak of Q fever in a rendering plant was traced to the processing of discarded guinea pigs infected with *C burnetii* (Feldman et al, 1950), and infections in laundry workers have been traced to the handling of contaminated clothing received from experimental laboratories (Oliphant et al, 1949)

REFERENCES

(For complete references, see also 2nd edition, Chapter 38)

Abinanti, F R, Lennette, E H, Winn, J F, and Welsh, H H, 1953a, Q fever studies XVIII Presence of *Coxiella burnetii* in the birth fluids of naturally-infected sheep Am J Hyg *58*, 385-388

Abinanti, F R, Welsh, H H, Lennette, E H, and Brunetti, O, 1953b, Q fever studies XVI Some aspects of the experimental infection induced in sheep by the intratracheal route of inoculation Am J Hyg *57*, 170-184

Abinanti, F R, Welsh, H H, Winn, J F, and Lennette, E H, 1955, Q fever studies XIX Presence and epidemiologic significance of *Coxiella burnetii* in sheep wool Am J Hyg *61*, 362 370

Babudieri, B, 1953a, Epidemiology, diagnosis and prophylaxis of Q fever *in* Advances in the Control of Zoonoses (Monograph Series), pp 157-174, Geneva, World Health Organ

———, 1953b, Laboratory techniques for diagnosis of Q fever *in* Advances in the Control of Zoonoses (Monograph Series), pp 193-210, Geneva, World Health Organ

Babudieri, B, and Secchi, P, 1952, La reazione di agglutinazione nella serodiagnosi dell infezione da Coxiella burneti, Rendic Ist super san *15*, 585-608

Beck, M D, Bell, J A, Shaw, E W, and Huebner, R J, 1949, Q fever studies in southern California II An epidemiological study of 300 cases Pub Health Rep *64*, 41-56

Bell, J A, Beck, M D, and Huebner, R J, 1950, Epidemiologic studies of Q fever in southern California, J A M A *142*, 868 872

Bengtson, I A, 1941a, Immunological relationships between the rickettsiae of Australian and American "Q" fever, Pub Health Rep *56*, 272-281

———, 1941b, Complement fixation in Q fever, Proc Soc Exper Biol & Med *46*, 665-668

Berge, T O, and Lennette, E H, 1953a, World distribution of Q fever Human, animal and arthropod infection Am J Hyg *57*, 125-143

———, 1953b, Q fever A study of serological relationships among strains of *Coxiella burnetii* (Derrick) Am J Hyg *57*, 144-169

Bickell, G, and Plattner, H, 1951, La terramycine en thérapeutique, Schweiz med Wchnschr *81*, 1-4

Blanc, G and Bruneau, J, 1949, Un reservoir de virus de la "Q Fever" en Algérie, la tique bovine "Hyalomma mauritanicum," Bull Acad nat med *133*, 115-117

Blanc, G, Bruneau, J, Poitrot, R, and Delage, B, 1948, Quelques donnees sur la Q fever (maladie de Derrick Burnet) experimentale, Bull Acad nat med *132*, 243-250

Carley, J G, and Pope, J H, 1953, The isolation of *Coxiella burneti* from the tick *Ixodes holocyclus* in Queensland, Australian J Exper Biol & Med *31*, 613-614

Clark, W H, Bogucki, A S, Lennette, E H, Dean B H, and Walker, J R, 1951a, Q fever in California VI Description of an epidemic occurring at Davis, California, in 1948 Am J Hyg *54*, 15-24

Clark, W H, and Lennette, E H, 1952, Treatment of Q fever with antibiotics, Ann New York Acad Sc *55*, 1004 1018

Clark, W H, Lennette, E H, and Meiklejohn, G, 1951b Q fever in California III Aureomycin in the therapy of Q fever Arch Int Med *87*, 204-217

Clark, W H, Lennette, E H, Railsback, O C, and Romer, M S, 1951c, Q fever in California VII Clinical features in one hundred eighty cases Arch Int Med *88*, 155-167

Clark, W H, Lennette, E H, and Romer, M S, 1951d, Q fever in California IX An outbreak aboard a ship transporting goats Am J Hyg *54*, 35-43

———, 1951e, Q fever in California XI An epidemiologic summary of 350 cases occurring in northern California during 1948-1949 Am J Hyg *54*, 319-330

Clark, W H, Romer, M S, Holmes, M A, Welsh, H H, Lennette, E H, and Abinanti, F R, 1951f, Q fever in California VIII An epidemic of Q fever in a small rural community in northern California Am J Hyg *54*, 25-34

Commission on Acute Respiratory Diseases, 1946a, Epidemics of Q fever among troops returning from Italy in the spring of 1945 II Epidemiological studies Am J Hyg *44*, 88-102

dicated by the fact that contaminated apparel has been incriminated epidemiologically as the source of rickettsiae in a number of human infections (Beck et al, 1949; Oliphant et al, 1949; Clark et al, 1951e, Derrick, 1953). Similarly, Q fever has occurred in wool- and hair-processing plants (Sigel et al, 1950) and the *importance of fleeces and hides in carrying the infection to the individual* is amply demonstrated by isolation of the rickettsia from sheep wool [illegible]
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Marmion, 1955)

CONTROL MEASURES

Satisfactory control of Q fever with respect to both the individual and the community still remains to be achieved Since the large preponderance of human infections appears to be acquired through direct or indirect contact with livestock, it would appear desirable to eliminate infection from herds or flocks However, since infection in livestock is of no consequence economically, it does not seem probable that any great effort would be made by animal husbandrymen to stamp out infection among their herds of cattle or goats or flocks of sheep through the administration of specific antibiotics or through vaccination even were the latter procedures effective and feasible economically Nor does it seem likely that any effective attempts will be undertaken in the near future to confine calving, kidding or lambing operations to a restricted area which can be decontaminated after the placenta and the dejecta have been collected and disposed of appropriately Consequently, to protect those whose occupation brings them into contact, however indirect, with livestock —for example, slaughterhouse workers, rendering-plant workers, cow and goat dairy workers, sheep and goat herders and other persons exposed to infective aerosols and dusts—immunization by means of a specific vaccine would appear to be desirable Meiklejohn and Lennette (1950) reported that no cases of clinical Q fever occurred among 50 persons vaccinated in their laboratory, further evidence of a protective effect is indicated by the discovery that 6 of the 50 individuals became infected, but no overt manifestations were elicited. The studies of Tigertt and Benenson (1956) provide somewhat more direct evidence of the efficacy of Q fever vaccines Eight individuals who had received vaccine prepared from the Henzerling strain were subsequently exposed to a moderate inhalatory dose of *C. burnetii* At the time of the exposure the antibody levels of these individuals were declining; the inhalatory exposure did not result in clinical disease but did produce a rise in the declining antibody levels Other individuals were similarly exposed 9 months after their initial vaccination and were found to be resistant to infection. Even those individuals who had not developed demonstrable complement-fixing antibodies at any time after vaccination were protected against respiratory inoculation Finally, to ascertain what effect administration of vaccine during the incubation period might have on the development of the disease, 5 volunteers were given 1 ml of vaccine 24 hours after respiratory exposure Overt illness developed in only 1 of the 5 individuals and then after a prolongation of the incubation period

In areas where Q fever is enzootic in domestic livestock, milk from cows, goats and sheep should be boiled or pasteurized Pasteurization by the vat-holding method (143° F. for 30 minutes) has been found inadequate to destroy *C burnetii* (Huebner et al, 1949b, Marmion et al, 1951; Lennette et al, 1952a). The comprehensive studies of Enright et al (1957) show that while a temperature of 143° F. for 30 minutes is inadequate to eliminate viable *C. burnetii* from milk, a temperature of 145° F. for 30 minutes ensures destruction of the rickettsia On the basis of their findings, these workers recommend that the requirements for high temperature short time (HTST) pasteurization be held at the present standard, viz, 161° F for 15 seconds

The possibility of person-to-person transmission of Q fever is so small that the quarantining of patients and the employment of extensive isolation precautions are not war-

ranted However, sterilization of sputum and excreta is recommended, and precautions should be taken in the handling, or the postmortem examination, of individuals dead of Q fever These recommendations are based on such episodes as that described by Siegert et al (1950) in which a hospitalized patient infected 38 other individuals, including patients and members of the staff Marmion and Stoker (1950) report several cases which occurred in connection with an autopsy, and Babudieri (1953a) mentions an episode in which 16 of the 17 individuals present at the postmortem on an individual who died of Q fever were infected

Care must be exercised in other respects also, and material which can even remotely be considered as contaminated should be sterilized Thus, an outbreak of Q fever in a rendering plant was traced to the processing of discarded guinea pigs infected with *C burnetii* (Feldman et al, 1950), and infections in laundry workers have been traced to the handling of contaminated clothing received from experimental laboratories (Oliphant et al, 1949)

REFERENCES

(For complete references, see also 2nd edition, Chapter 38)

Abinanti, F R, Lennette, E H, Winn, J F, and Welsh, H H, 1953a, Q fever studies XVIII Presence of *Coxiella burnetii* in the birth fluids of naturally-infected sheep Am J Hyg *58*, 385-388

Abinanti, F R, Welsh, H H, Lennette, E H, and Brunetti, O, 1953b, Q fever studies XVI Some aspects of the experimental infection induced in sheep by the intratracheal route of inoculation Am J Hyg *57*, 170 184

Abinanti, F R, Welsh, H H, Winn, J F, and Lennette, E H, 1955, Q fever studies XIX Presence and epidemiologic significance of *Coxiella burnetii* in sheep wool Am J Hyg *61*, 362 370

Babudieri, B, 1953a, Epidemiology, diagnosis and prophylaxis of Q fever *in* Advances in the Control of Zoonoses (Monograph Series), pp 157-174, Geneva, World Health Organ

———, 1953b, Laboratory techniques for diagnosis of Q fever *in* Advances in the Control of Zoonoses (Monograph Series), pp 193-210, Geneva, World Health Organ

Babudieri, B, and Secchi, P, 1952, La reazione di agglutinazione nella serodiagnosi dell infezione da Coxiella burneti, Rendic Ist super san *15*, 585-608

Beck, M D, Bell, J A, Shaw, E W, and Huebner, R J, 1949, Q fever studies in southern California II An epidemiological study of 300 cases Pub Health Rep *64*, 41-56

Bell, J A, Beck, M D, and Huebner, R J, 1950, Epidemiologic studies of Q fever in southern California, J.A.M.A *142*, 868-872

Bengtson, I A, 1941a, Immunological relationships between the rickettsiae of Australian and American "Q" fever, Pub Health Rep *56*, 272-281

———, 1941b, Complement fixation in Q fever, Proc Soc Exper Biol & Med *46*, 665-668

Berge, T O, and Lennette, E H, 1953a, World distribution of Q fever Human, animal and arthropod infection Am J Hyg *57*, 125-143

———, 1953b, Q fever A study of serological relationships among strains of *Coxiella burnetii* (Derrick) Am J Hyg *57*, 144-169

Bickell, G, and Plattner, H, 1951, La terramycine en therapeutique, Schweiz med Wchnschr *81*, 1-4

Blanc, G, and Bruneau, J, 1949, Un réservoir de virus de la "Q Fever" en Algerie, la tique bovine "Hyalomma mauritanicum," Bull Acad nat med *133*, 115-117

Blanc, G, Bruneau, J, Poitrot, R, and Delage, B, 1948, Quelques donnees sur la Q fever (maladie de Derrick-Burnet) experimentale, Bull Acad nat med *132*, 243-250

Carley, J G, and Pope, J H, 1953, The isolation of *Coxiella burneti* from the tick *Ixodes holocyclus* in Queensland, Australian J Exper Biol & Med *31*, 613-614

Clark, W H, Bogucki, A S, Lennette, E H, Dean, B H, and Walker, J R, 1951a, Q fever in California VI Description of an epidemic occurring at Davis, California, in 1948 Am J Hyg *54*, 15-24

Clark, W H, and Lennette, E H, 1952, Treatment of Q fever with antibiotics, Ann New York Acad Sc *55*, 1004 1018

Clark, W H, Lennette, E H, and Meiklejohn, G, 1951b, Q fever in California III Aureomycin in the therapy of Q fever Arch Int Med *87*, 204-217

Clark, W H, Lennette, E H, Railsback, O C, and Romer, M S, 1951c, Q fever in California VII Clinical features in one hundred eighty cases Arch Int Med *88*, 155-167

Clark, W H, Lennette, E H, and Romer, M S, 1951d, Q fever in California IX An outbreak aboard a ship transporting goats Am J Hyg *54*, 35-43

———, 1951e, Q fever in California XI An epidemiologic summary of 350 cases occurring in northern California during 1948-1949 Am J Hyg *54*, 319-330

Clark, W H, Romer, M S, Holmes, M A, Welsh, H H, Lennette, E H, and Abinanti, F R, 1951f, Q fever in California VIII An epidemic of Q fever in a small rural community in northern California Am J Hyg *54*, 25-34

Commission on Acute Respiratory Diseases, 1946a, Epidemics of Q fever among troops returning from Italy in the spring of 1945 II Epidemiological studies Am J Hyg *44*, 88-102

dicated by the fact that contaminated apparel has been incriminated epidemiologically as the source of rickettsiae in a number of human infections (Beck et al., 1949; Oliphant et al, 1949; Clark et al., 1951e; Derrick, 1953). Similarly, Q fever has occurred in wool- and hair-processing plants (Sigel et al, 1950) and the importance of fleeces and hides in carrying the infection to the individual is amply demonstrated by isolation of the rickettsia from sheep wool (Abinanti et al, 1955, Stoker et al, 1955a). Contaminated microenvironments explain not only the occurrence of such cases but also those which occurred in laundries, an art school, a locksmith shop (cf. Welsh et al, 1957), and among personnel handling mail bags carried in vehicles previously used for the transportation of animals, and also among persons exposed to dust from grain used as animal food (cf Stoker and Marmion, 1955)

CONTROL MEASURES

Satisfactory control of Q fever with respect to both the individual and the community still remains to be achieved Since the large preponderance of human infections appears to be acquired through direct or indirect contact with livestock, it would appear desirable to eliminate infection from herds or flocks However, since infection in livestock is of no consequence economically, it does not seem probable that any great effort would be made by animal husbandrymen to stamp out infection among their herds of cattle or goats or flocks of sheep through the administration of specific antibiotics or through vaccination even were the latter procedures effective and feasible economically Nor does it seem likely that any effective attempts will be undertaken in the near future to confine calving, kidding or lambing operations to a restricted area which can be decontaminated after the placenta and the dejecta have been collected and disposed of appropriately Consequently, to protect those whose occupation brings them into contact, however indirect, with livestock —for example, slaughterhouse workers, rendering-plant workers, cow and goat dairy workers, sheep and goat herders and other persons exposed to infective aerosols and dusts—immunization by means of a specific vaccine would appear to be desirable Meiklejohn and Lennette (1950) reported that no cases of clinical Q fever occurred among 50 persons vaccinated in their laboratory, further evidence of a protective effect is indicated by the discovery that 6 of the 50 individuals became infected, but no overt manifestations were elicited. The studies of Tigertt and Benenson (1956) provide somewhat more direct evidence of the efficacy of Q fever vaccines. Eight individuals who had received vaccine prepared from the Henzerling strain were subsequently exposed to a moderate inhalatory dose of *C burnetii* At the time of the exposure the antibody levels of these individuals were declining; the inhalatory exposure did not result in clinical disease but did produce a rise in the declining antibody levels Other individuals were similarly exposed 9 months after their initial vaccination and were found to be resistant to infection Even those individuals who had not developed demonstrable complement-fixing antibodies at any time after vaccination were protected against respiratory inoculation Finally, to ascertain what effect administration of vaccine during the incubation period might have on the development of the disease, 5 volunteers were given 1 ml of vaccine 24 hours after respiratory exposure Overt illness developed in only 1 of the 5 individuals and then after a prolongation of the incubation period

In areas where Q fever is enzootic in domestic livestock, milk from cows, goats and sheep should be boiled or pasteurized Pasteurization by the vat-holding method (143° F. for 30 minutes) has been found inadequate to destroy *C burnetii* (Huebner et al., 1949b; Marmion et al, 1951, Lennette et al, 1952a) The comprehensive studies of Enright et al. (1957) show that while a temperature of 143° F. for 30 minutes is inadequate to eliminate viable *C. burnetii* from milk, a temperature of 145° F. for 30 minutes ensures destruction of the rickettsia On the basis of their findings, these workers recommend that the requirements for high temperature short time (HTST) pasteurization be held at the present standard, viz, 161° F. for 15 seconds

The possibility of person-to-person transmission of Q fever is so small that the quarantining of patients and the employment of extensive isolation precautions are not war-

armion, [redacted] P, and Stoker, [redacted] G P, 1950, Q fever in Great Britain Epidemiology of an outbreak. Lancet 2, 611-616

eiklejohn, G, and Lennette, E H, 1950, Q fever in California I. Observations on vaccination of

J Hyg 49, 76-82

arker, R R, and Steinhaus, [redacted] A, 1943, American and Australian [redacted] fevers persistence of the infectious agents in guinea pig tissues after defervescence, Pub Health Rep 58, 523-527

erez Gallardo, F, Clavero, G, and Hernández, S, 1949, Hallazgo en España de la "Rickettsia burnetii" agente etiologico de la fiebre "Q." Rev san [redacted] hig pub 23, 489-496

——, 1952, Investigaciones sobre la epidemiologia de la fiebre "Q" en España Los conejos de monte y los lirones como reservorios de la "Coxiella burnetii," Rev san e hig pub 26, 81-87

errin, T L, 1949, Histopathologic observations in a fatal case of Q fever, Arch Path 47, 361-365

hilip, C B, 1948, Comments on the name of the Q fever organism, Pub Health Rep 63, 58

aška, K, and Syruček, L, 1956, Ein Beitrag zur Epidemiologie der Q-Rickettsiose, Zentralbl Bakt 167, 267-280

obbins, F C, Gauld, R L, and Warner, F B, 1946a, Q fever in the Mediterranean area report of its occurrence in Allied troops II Epidemiology Am J Hyg 44, 23-50

obbins, [redacted] C, and Rustigian, R, 1946, Q fever in the Mediterranean area report of its occurrence in Allied troops IV A laboratory outbreak Am J Hyg 44, 64-71

Robbins, F C, Rustigian, R, Snyder, M J, and Smadel, J E, 1946b, Q fever in the Mediterranean area report of its occurrence in Allied troops III The etiological agent Am J Hyg 44, 51-63

Shepard, C C, 1947, An outbreak of Q fever in a Chicago packing house, Am J Hyg 46, 185-192

Shepard, C C, and Huebner, [redacted] J, 1948, Q fever in Los Angeles County, description of some of its epidemiological features, Am J Pub Health 38, 781-788

Siegert, R, Simrock, W, and Stroeder, U, 1950, Über einen epidemischen Ausbruck von Q-Fieber in einem Krankenhaus, Ztschr Tropenmed u Parasitol 2, 1-40

Sigel, M M, Scott, T F McN, Henle, W, and Janton, O H, 1950, Q fever in a wool and hair processing plant, Am J Pub Health 40, 524-532

Smadel, J E, Jackson, E B, and Cruise, A B, 1949, Chloromycetin in experimental rickettsial infections, J Immunol 62, 49-65

Smadel, J E, Jackson, E B, and Ley, H L, Jr, 1950, Terramycin [redacted] a rickettsiostatic agent and its usefulness in patients with scrub typhus, Ann New York Acad Sc 53, 375 384

Smadel, J E, Snyder, M J, and Robbins, [redacted] C, 1948, Vaccination against Q fever, Am J Hyg 47, 71-81

Spicknall, C G, Huebner, R J, Finger, J A, and Blocker, W P, 1947, Report of an outbreak of Q fever at the National Institute of Health I Clinical features Ann Int Med 27, 28-40

Stoker, M G P, Brown, [redacted] D, Kett, F J L, Collings, [redacted] C, and Marmion, B P, 1955a, Q fever in Britain isolation of *Rickettsia burneti* from placenta and wool of sheep in an endemic area, J Hyg 53, 313-321

Stoker, M G P, and Marmion, B P, 1955, The spread of Q fever from animals to man The natural history of a rickettsial disease Bull World Health Organ 13, 781-806

Stoker, M G P, Page, Z, and Marmion, [redacted] P, 1955b, Problems in the diagnosis of Q fever by complement-fixation tests, Bull World Health Organ 13, 807-827

Syruček, L, and Raška, K, 1956, Q fever in domestic and wild birds, Bull World Health Organ 15, 329-337

Tigertt, W D, and Benenson, A S, 1956, Studies on Q fever in man, Tr Assn Am Physicians 69, 98-104

Topping, [redacted] H, Shepard, C C, and Huebner, R J, 1946, Q fever an immunological comparison of strains, Am J Hyg 44, 173-182

Topping, [redacted] H, Shepard, C C, and Irons, J V, 1947, Q fever in the United States I Epidemiologic studies of an outbreak among stock handlers and slaughterhouse workers J.A.M.A 133, 813-815

Wegmann, T, 1948, Über eine Q-Fever (Queensland fieber) Epidemie in Graubünden, Schweiz med Wchnschr 78, 529-531

Welsh, H H, Lennette, E H, Abinanti, F R, and Winn, J F, 1951, Q fever in California IV Occurrence of *Coxiella burnetii* in the placenta of naturally infected sheep Pub Health Rep 66, 1473-1477

——, 1958, Airborne transmission of Q fever The role of parturition in the generation of infective aerosols Ann New York Acad Sc 70, 528-540

Weyer, F, 1953, Die Beziehungen des Q-Fieber-Erregers (Rickettsia burneti) zu Arthropoden, Ztschr Tropenmed [redacted] Parasitol 4, 344-382

Whittick, J W, 1950, Necropsy findings in a case of Q fever in Britain, Brit Med J 1, 979 980

Winn, J F, Lennette, E H, Welsh, H H, and Abinanti, F R, 1953, Q fever studies XVII Presence of *Coxiella burnetii* in the feces of naturally infected sheep Am J Hyg 58, 183-187

Ze

ver Report of a case treated with chloromycetin, Ann Int Med 32, 982-987

Commission on Acute Respiratory Diseases, 1946b, Identification and characteristics of the Balkan grippe strain of *Rickettsia burneti*, Am J Hyg *44*, 110-122

———, 1946c, A laboratory outbreak of Q fever caused by the Balkan grippe strain of *Rickettsia burneti*, Am. J. Hyg. *44*, 123-157

Cox, H R., 1940, *Rickettsia diaporica* and American Q fever, Am J. Trop Med. *20*, 463-469

Cox, H R, Tesar, W. C, and Irons, J. V., 1947, Q fever in the United States IV Isolation and identification of rickettsias in an outbreak among stock handlers and slaughterhouse workers J.A.M.A *133*, 820-821

De Lay, P D, Lennette, E H, and De Ome, K B, 1950, Q fever in California Recovery of *Coxiella burnetii* from naturally-infected air-borne dust J Immunol *65*, 211-220

Derrick, E H, 1944, The epidemiology of Q fever, J Hyg *43*, 357-361

———, 1953, The epidemiology of "Q" fever a review Med J Australia *1*, 245-253

Dyer, R E, Topping, N H, and Bengtson, I A, 1940, An institutional outbreak of pneumonitis II Isolation and identification of causative agent Pub Health Rep *55*, 1945-1954

Enright, J B, Sadler, W W, and Thomas, R C, 1957, Thermal inactivation of *Coxiella burnetii* and its relation to pasteurization of milk Public Health Monograph No 47, issued concurrently with Pub Health Rep *72*, No 10 (October)

Feinstein, M, Yesner, R, and Marks, J L, 1946, Epidemics of Q fever among troops returning from Italy in the Spring of 1945 I Clinical aspects of the epidemic at Camp Patrick Henry, Virginia Am J Hyg *44*, 72-87

Feldman, H A, Silverman, A C, and Adair, C V, 1950, An epidemic of Q fever among employees of a rendering plant in Syracuse, New York, J Clin Invest (Abstract) *29*, 812.

Fonseca, F., Pinto, M R, Oliveira, J, da Gama, M M, and Lacerda, M T, 1949, Febre Q em Portugal, Clin contemp *3*, 1567-1578

Harman, J B, 1949, Q fever in Great Britain Clinical account of 8 cases Lancet *2*, 1028-1030

Hornibrook, J. W, and Nelson, K R, 1940, An institutional outbreak of pneumonitis I Epidemiological and clinical studies. Pub Health Rep *55*, 1936-1944.

Huebner, R J., 1947, Report of an outbreak of Q fever at National Institute of Health II Epidemiological features Am J Pub Health *37*, 431-440

Huebner, R J, Jellison, W L, and Beck, M D, 1949a, Q fever—a review of current knowledge, Ann Int Med *30*, 495-509.

Huebner, R J, Jellison, W L, Beck, M D, Parker, R. R, and Shepard, C C, 1948, Q fever studies in southern California I Recovery of *Rickettsia burneti* from raw milk Pub Health Rep *63*, 214-222

Huebner, R J, Jellison, W L, Beck, M. D, and Wilcox, F. P., 1949b, Q fever studies in southern California III Effects of pasteurization on survival of *Coxiella burneti* in naturally infected milk Pub Health Rep *64*, 499-511

Jacobson, G., Denlinger, R. B, and Carter, R. A, 1949, Roentgen manifestations of Q fever, Radiology *53*, 739-749.

Kaplan, M. M, and Bertagna, P, 1955, The geographical distribution of Q fever, Bull World Health Organ. *13*, 829-860

Kikuth, W., and Bock, M, 1949, Drei-und-zwanzig Falle von Laboratoriums-infektionen mit Q fieber, Med Klin. *44*, 1056-1060.

Lennette, E. H, 1956, Q fever *in* Meakins, J. C. (ed), The Practice of Medicine, ed 6, pp 469-473, St Louis, Mosby.

Lennette, E. H., and Clark, W. H, 1951, Observations on the epidemiology of Q fever in northern California, J.A.M.A *145*, 306-309.

Lennette, E H, Clark, W. H., Abinanti, M. M, Brunetti, O, and Covert, J. M., 1952a, Q fever studies. XIII. The effect of pasteurization on *Coxiella burnetii* in naturally infected milk Am J. Hyg *55*, 246-253

Lennette, E. H, Clark, W. H., and Dean, B H, 1949, Sheep and goats in the epidemiology of Q fever in northern California, Am J Trop Med *29*, 527-541

Lennette, E. H, Clark, W H., Jensen, F W, and Toomb, C J, 1952b, Q fever studies XV Development and persistence in man of complement-fixing and agglutinating antibodies to *Coxiella burnetii* J. Immunol *68*, 591-598

Lennette, E H, Holmes, M. A, and Abinanti, F R, 1952c, Q fever studies XIV Observations on the pathogenesis of the experimental infection induced in sheep by the intravenous route Am J Hyg *55*, 254-267

Lennette, E H, and Meiklejohn, G, 1948, Q fever in central and northern California, California Med *69*, 197-199

in California X Recovery of *Coxiella burnetii* from the air of premises harboring infected goats Am J Hyg *54*, 44-49

Lillie, R D, 1942, Pathologic histology in guinea pigs following intraperitoneal inoculation with the virus of Q fever, Pub Health Rep *57*, 296-306

Lillie, R D, Perrin, T L, and Armstrong, C, 1941, An institutional outbreak of pneumonitis III Histopathology in man and rhesus monkeys of the pneumonitis due to the virus of Q fever Pub Health Rep *56*, 149-155

Luoto, L, 1956, A capillary agglutination test for antibody against *Coxiella burnetii* in human, guinea pig and sheep sera, J. Immunol *77*, 294-298

Luoto, L, and Huebner, R J., 1950, Q fever studies in southern California IX Isolation of Q fever organisms from parturient placentas of naturally infected dairy cows Pub Health Rep *65*, 541-544

Marmion, B P, MacCallum, F O, Rowlands, A, and Thiel, C. C, 1951, The effect of pasteurization on milk containing *Rickettsia burnetii*, Month Bull Min Health *10*, 119-128

with undermined, irregular edges Eventually healing is complete without scar formation (Flaum, 1939)

The histopathologic picture of experimentally induced lesions consists of hyperkeratosis, swollen and hyperplastic cells of the malpighian layer which disintegrate to form loculated vesicles, intranuclear inclusions in cells near the vesicles, and infiltration of adjacent tissues with polymorphonuclear leukocytes The disease can be transmitted experimentally to the guinea pig by cutaneous and subcutaneous inoculation of the foot pads, and to suckling mice by intraperitoneal inoculation Necrosis of skeletal muscle occurs in young mice Rabbits, rats, mice, dogs and cats are irregularly susceptible while fowl (except ducks), ferrets and horses are usually resistant The resistance of the horse is of prime importance, for it serves to differentiate the virus of foot-and-mouth disease from that of equine vesicular stomatitis

The diameter of the virus as determined by filtration through gradocol membranes is 10 to 12 mμ, while electronmicroscopy and ultracentrifugation indicate the average particle size to be larger, 20 to 30 mμ The complement-fixing antigen measures 6 to 7 mμ The virus in tissues is fairly resistant to disinfectants, the most practicable virucidal agent is 0 5 to 2 per cent sodium hydroxide (Olitsky et al, 1928) The active agent is stable in 2 pH zones, namely, 2 5 to 3 5 and 6 5 to 10 0 It can be grown in tissue cultures only when tissue from susceptible species is used There are 7 immunologically distinct types During convalescence, human beings and lower animals produce antibodies specific for the particular strain inducing the infection

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Following the mass cultivation of the viruses in explants of cattle tongue epithelium (Frenkel, 1950) this and other types of bovine tissue culture have been employed for studies of viral multiplication (Sellers, 1955, Cartwright et al, 1957) and for the manufacture of formaldehyde-inactivated vaccine Unfortunately, the best vaccines produce an immunity which lasts only 4 to 6 months, and repeated vaccination is necessary

NEWCASTLE DISEASE

(SYNONYMS Avian pseudoplague, avian pneumoencephalitis)

Newcastle disease (ND) is an epizootic infection of fowl characterized by viremia and signs of involvement of the respiratory, the gastro-intestinal and the central nervous systems The disease has been recognized as a distinct clinical and pathologic entity since the work of Doyle, 1927, and has been encountered in continental Europe, England, Asia, Africa and Australia It was first recognized in the United States in California as pneumoencephalitis, Beach, 1946, and is known to have reached the East coast by 1944 Beach, 1944, showed that the viruses of Newcastle disease and pneumoencephalitis are immunologically identical There is an opinion that pneumonic and nervous signs dominate the American type of infection, while respiratory and gastro-intestinal signs are the most obvious in the Old World type, Brandly et al, 1946a The malady occasionally attacks human beings who handle infected fowl or work with the ND virus, manifesting itself chiefly as a superficial conjunctivitis At least 10 proved and 17 suspected cases have been reported

Newcastle disease in fowl usually has an incubation period of 4 to 11 days Onset is sudden with drowsiness, rapid respiration and fever Then, diarrhea sets in, brown fluid or saliva drools from the beak, a thick mucous discharge from the nose appears, and respiratory distress, cyanosis and petechiae of the wattles and the comb occur Opisthotonos, convulsions, ascending paresis and abnormal movements have also been observed Death ensues, usually on the 6th to the 8th day, in

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46

Infections of Minor Importance

This chapter is concerned with several virus diseases with well-characterized agents as well as certain infectious processes in which a viral etiology is only presumptive Certain of them e g , trench fever and viral gastroenteritis, usually occur as epidemics Others represent diseases of animals in which human infection is accidental Still others, like warts, are extremely common in man but rarely produce serious illness In a few instances, as encephalitis lethargica, diseases are discussed which, although major medical problems at one time, are now almost extinct

A *Viral Diseases With Primary Reservoir in Nonhuman Hosts*
 1 Foot-and-Mouth Disease
 2 Newcastle Disease
 3 Equine Infectious Anemia
 4 Cat-Scratch Disease
 5 Lymphocytic Choriomeningitis
 6. Encephalomyocarditis

B *Viral Disease With Reservoir Unknown*
 1 Warts. Verrucae
 2 Molluscum Contagiosum
 3 Epidemic Viral Gastroenteritis
 4. Salivary Gland Virus Infection

C *Diseases of Presumptive Viral or Rickettsial Origin*
 1. Infectious Polyneuritis
 2. Hemorrhagic Meningoencephalitis
 3. Durand's Disease
 4 Encephalitis Lethargica
 5 Postinfection (Demyelinating) Encephalitis
 6 Trench Fever

FOOT-AND-MOUTH DISEASE

(SYNONYMS *Fievre aphtheuse, Maul-und-Klauenseuche,* aphthous fever, epizootic *aphthae, epizootic stomatitis*)

Foot-and-mouth disease is a highly contagious infection of cloven-footed animals, especially of cattle, pigs, sheep and goats, and is rarely transmitted to human beings, who become infected by ingestion of virus-contaminated food, by handling the active agent, or through contact with affected animals Since epizootics of the disease are rare in the United States, the malady in human beings in this country is uncommon. However, the disease is now endemic in Mexico and Canada

The disease in lower animals is characterized by fever, salivation and the appearance of large, coalescing vesicles in the mucous membranes of the mouth, the tongue and the lips, and in the skin of the hoofs, dewclaws, interdigital areas and the udder The disease in human beings manifests itself, after an incubation period of 2 to 18 days, by fever, salivation, vesicles in the mucous membranes of the mouth, the pharynx, the lips and the tongue and in the skin of the soles, the palms, the digits and the interdigital areas Within a few days, the vesicles rupture, leaving ulcers

with undermined, irregular edges Eventually healing is complete without scar formation (Flaum, 1939)

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the United States the mortality rate is comparatively low in adult birds. The disease in fowl produces multiple focal necroses in the viscera and hemorrhages, especially in the respiratory and the alimentary tracts; at times an interstitial pneumonitis is observed. The CNS may show localized meningo-encephalitis characterized by areas of necrosis of the ground substance, neuronal necrosis and degeneration, and small hemorrhages.

The proved cases in man occurred in laboratory or poultry workers handling the virus, in whom the incubation period varied from a few hours to 2 days The disease manifests itself as a unilateral superficial conjunctivitis without involvement of the cornea; or a syndrome, consisting of conjunctivitis, preauricular lymphadenitis, headache, malaise and chills, without significant rise in temperature, may occur All reported patients have recovered completely within 1 or 2 weeks.

The natural disease is observed in chickens, turkeys, pheasants, guinea fowl, sparrows, crows, francolins and parrots; experimental infection has been achieved in chick embryos, ducks, geese, pigeons and several varieties of wild birds Intracerebral inoculation of the virus into mice, hamsters, cotton rats and rhesus monkeys causes a meningo-encephalitis which becomes increasingly severe with repeated passage and adaptation to these hosts (Wenner et al, 1950) Large doses of virus given to mice intranasally produce consolidation of the lungs, but it is not possible to transfer the disease from mouse to mouse in this manner Ferrets show no apparent malady after intranasal application of the active agent but develop specific antibody Guinea pigs, *rabbits and pigs are resistant. Transmission* of the disease can be effected by means of blood, brain, viscera, oral and nasal secretions, and feces All routes of inoculation, except the intramuscular which may be followed by irregular results, can be employed successfully. The diameter of ND virus as determined by filtration through gradocol membranes is 80 to 120 mμ; 115 mμ (Bang, 1946) by elecronmicrography

The virus particle exists in spherical and filamentous forms which like influenza virus,
rimentally
The aver-
0 mμ and

that of the filaments 90 mμ. The length of the latter form varies between 270 and 980 mμ (Kilham et al., 1951). It is said to be filterable through Berkefeld V, N and W candles, Chamberland L_3 and L_5 filters and Seitz pads It is inactivated at 60° C for 30 minutes and at 55° C. for 45 minutes by photodynamic action of methylene blue, by ultraviolet radiation, by 1:5,000 dilution of formalin, and by N/50 sodium hydroxide in 1 hour but not by N/25 hydrochloric acid Newcastle virus remains active at pH 4 for at least a week (Brandly et al, 1946). The virus is preserved in 50 per cent glycerol, by being kept frozen at −70° C., and by lyophilization.

Newcastle disease virus multiplies readily in embryonated eggs and in cultures of chick *embryonic tissues or HeLa cells (Tyrrell,* 1955). Cellular destruction is produced in such tissue cultures. Allantoic fluid from infected embryos will fix complement with immune serum, will hemolyse chicken erythrocytes and is capable of causing hemagglutination of fowl erythrocytes and those of certain other species (Burnet, 1943). By centrifugation it is possible to separate a large (L) and a small (S) hemagglutinating component which have differing elution properties (Granoff and Henle, 1954). Hemolytic activity appears to be associated with the L component only ND virus hemagglutination is inhibited by serum antibody, and this has made possible in vitro technics for measuring the concentrations of viruses or antibody. Human erythrocytes sensitized with ND virus are agglutinated by specific antisera and by the sera of some patients with infectious mononucleosis (Burnet and Anderson, 1946, Florman, *1949, Evans, 1955) An antigenic relationship* to mumps was suggested by the observation by Jungherr et al. (1949) that mumps convalescents may develop neutralizing antibody to Newcastle disease virus However, as these studies were performed with unheated sera, which might have contained nonspecific inhibitors (see below), and since

susceptible lower animals. They are produced

also by animals which are resistant to infection following parenteral injection of the virus Specific antibodies can be detected, and their titer can be determined by means of the hemagglutination-inhibition test, the virus-neutralization test in chick embryos, or the complement-fixation test

The diagnosis of Newcastle disease in human beings is made by: (1) a history of exposure to the virus or to fowl ill with the disease, (2) isolation of the virus from the conjunctival exudate and its identification by means of the hemagglutination-inhibition test, and (3) determination of the development of specific neutralizing antibody by tests on paired sera collected from patients during the acute and the convalescent stages of the disease Because of the presence in human serum of a heat-labile nonspecific neutralizing factor it is essential that suspect sera be inactivated at 56° C for 30 minutes before being used in neutralization tests (Ginsberg and Horsfall, 1949, Howitt, 1950). No specific treatment is available Antibiotics have no effect on the virus

The few human cases reported point to the fact that exposure to the virus or to affected fowl is essential for infection In fowl the disease is ordinarily conveyed by contact A natural resistance develops with age, and passive immunity can be conferred upon a chick by way of the egg yolk of an immune dam, Brandly et al, 1946a The prevention of the disease in man depends on protection from infection when exposed to the virus or the affected fowl The prevention of the disease in birds has been shown to be possible through the use of formolized or irradiated vaccines, or, if a stronger and more durable immunity is desired, by the employment of such vaccines, followed by a vaccine consisting of modified, active virus, Brandly et al, 1946c

EQUINE INFECTIOUS ANEMIA

(SYNONYMS. Swamp fever of horses, pernicious anemia of horses, equine "malaria")

Equine infectious anemia is a viral disease of horses, mules and donkeys which is widely distributed over the world In equines it appears in an acute form enduring for 4 days to 3 weeks, a chronic type which may last for years accompanied by viremia or as a subacute, quiescent illness The mortality varies between 30 and 70 per cent The question of human susceptibility remains open since it has been reported as transmissible to man (Peters, 1945, Kral, 1949) while Stein et al (1944) and Dreguss and Lombard (1954) present considerable evidence against human infection The reported disease in man manifested itself by fever, anemia, diarrhea, renal pain and a "viremia" The blood of one person was reported as infective for horses over a period of 3 years

The virus was discovered by Vallée and Carré, 1904, its diameter as determined by filtration through gradocol membranes is 18 to 50 mμ, it is inactivated by heating to 60° C. for 1 hour. Equine animals are susceptible, while ordinary laboratory animals are resistant The observation by Reagan et al (1948) that rabbits develop a transmissible febrile illness following inoculation of infected horse blood awaits confirmation Transmission in equines can be effected by the parenteral injection of infectious material, by biting flies (*Stomoxys calcitrans*), or by virus coming in contact with abraded skin or mucous membranes (Stein et al, 1944). The idea, formerly held, that infection by ordinary contact or feeding is possible, has not been substantiated experimentally However, it has been demonstrated that infected mares may have virus in their milk and that suckling foals, although inapparently harboring the disease, may still be susceptible to experimental infection by a peripheral route There is no specific treatment, and control measures consist of isolation or extermination of affected animals. It is important to note that all antisera made in equine animals for use in man or equine animals must be produced in a manner to eliminate the possibility of their containing the virus of infectious anemia in an active state. The monograph of Dreguss and Lombard (1954) contains an extensive description of this disease and its bibliography.

CAT-SCRATCH DISEASE

(SYNONYMS. Cat-scratch fever, benign lymphoreticulosis)

Cat-scratch disease is a systemic illness, rarely fatal, characterized by malaise, fever, and a lymphadenitis which may resemble neo-

the United States the mortality rate is comparatively low in adult birds. The disease in fowl produces multiple focal necroses in the viscera and hemorrhages, especially in the respiratory and the alimentary tracts; at times an interstitial pneumonitis is observed. The CNS may show localized meningo-encephalitis characterized by areas of necrosis of the ground substance, neuronal necrosis and degeneration, and small hemorrhages

The proved cases in man occurred in laboratory or poultry workers handling the virus, in whom the incubation period varied from a few hours to 2 days. The disease manifests itself as a unilateral superficial conjunctivitis without involvement of the cornea, or a syndrome, consisting of conjunctivitis, preauricular lymphadenitis, headache, malaise and chills, without significant rise in temperature, may occur All reported patients have recovered completely within 1 or 2 weeks

The natural disease is observed in chickens, turkeys, pheasants, guinea fowl, sparrows, crows, francolins and parrots; experimental infection has been achieved in chick embryos, ducks, geese, pigeons and several varieties of wild birds Intracerebral inoculation of the virus into mice, hamsters, cotton rats and rhesus monkeys causes a meningo-encephalitis which becomes increasingly severe with repeated passage and adaptation to these hosts (Wenner et al, 1950). Large doses of virus given to mice intranasally produce consolidation of the lungs, but it is not possible to transfer the disease from mouse to mouse in

rabbits and pigs are resistant. Transmission of the disease can be effected by means of blood, brain, viscera, oral and nasal secretions, and feces All routes of inoculation, except the intramuscular which may be followed by irregular results, can be employed successfully. The diameter of ND virus as determined by filtration through gradocol membranes is 80 to 120 mμ; 115 mμ (Bang, 1946) by electronmicrography.

The virus particle exists in spherical and filamentous forms which, like influenza virus, develop from the surface of experimentally infected chick chorio-allantois cells The average diameter of the spheres is 100 mμ and that of the filaments 90 mμ. The length of the latter form varies between 270 and 980 mμ (Kilham et al., 1951). It is said to be filterable through Berkefeld V, N and W candles, Chamberland L_3 and L_5 filters and Seitz pads It is inactivated at 60° C. for 30 minutes and at 55° C. for 45 minutes by photodynamic action of methylene blue, by ultraviolet radiation, by 1:5,000 dilution of formalin, and by N/50 sodium hydroxide in 1 hour but not by N/25 hydrochloric acid. Newcastle virus remains active at pH 4 for at least a week (Brandly et al, 1946). The virus is preserved in 50 per cent glycerol, by being kept frozen at −70° C., and by lyophilization.

Newcastle disease virus multiplies readily in embryonated eggs and in cultures of chick embryonic tissues or HeLa cells (Tyrrell, 1955). Cellular destruction is produced in such tissue cultures. Allantoic fluid from infected embryos will fix complement with immune serum, will hemolyse chicken erythrocytes and is capable of causing hemagglutination of fowl erythrocytes and those of certain other species (Burnet, 1943). By centrifugation it is possible to separate a large (L) and a small (S) hemagglutinating component which have differing elution properties (Granoff and Henle, 1954). Hemolytic activity appears to be associated with the L component only. ND virus hemagglutination is inhibited by serum antibody, and this has made possible in vitro technics for measuring the concentrations of viruses or antibody. Human erythrocytes sensitized with ND virus are agglutinated by specific antisera and by the sera of some patients with infectious mononucleosis (Burnet and Anderson, 1946; Florman, 1949, Evans, 1955). An antigenic relationship to mumps was suggested by the observation by Jungherr et al. (1949) that mumps convalescents may develop neutralizing antibody to Newcastle disease virus However, as these studies were performed with unheated sera, which might have contained nonspecific inhibitors (see below), and since

lowing infection with the agent in man and susceptible lower animals. They are produced

pecially the mouse, in which the CNS and particularly the meninges and the choroid plexuses are involved, it is transmissible to man in whom the infection produces a marked diversity of signs and symptoms

History

The virus of lymphocytic choriomeningitis (LCM) was isolated from a monkey by Armstrong and Lillie in 1934 during the course of an investigation of St Louis encephalitis Shortly thereafter this agent was found in the cerebrospinal fluid of several human patients in whom a clinical diagnosis of benign aseptic meningitis had been made (Rivers and Scott, 1935) Lepine et al (1937) demonstrated that by subcutaneous injection of the virus from mice, performed as a therapeutic measure, LCM could be induced in human beings and that the disease was transmissible in man by means of intramuscular inoculations of infected blood Natural and laboratory infections are not uncommon, and these are discussed in the general review by Janeway and Farmer (1942) The intensive investigations of Traub (1936) focused attention on rodents, particularly mice, as both a vector and a reservoir of the virus of LCM

Clinical Picture

Wallgren (1925) used the term "acute aseptic meningitis" to designate a clinical syndrome in man that he thought was a nosologic entity. He described it as an acute febrile, nonfatal malady characterized by symptoms and signs of meningeal irritation and associated at times with infection of the upper respiratory tract Now it is known that acute aseptic meningitis is not a nosologic entity but represents a clinical syndrome which may be caused by more than one etiologic agent, one of which is the virus of lymphocytic choriomeningitis

Infection by this virus may assume a number of different clinical forms (Smadel et al, 1942, Adair et al, 1953) such as aseptic meningitis, grippe, meningo-encephalomyelitis and acute fatal systemic disease, often it is clinically inapparent. In most instances the meningeal and grippelike types prevail After an unknown period of incubation, the onset is sudden, frequently with symptoms and signs similar to those of influenza In many patients, this is all that happens, and recovery ensues promptly In others, the grippe phase is followed by definite signs of meningitis which may endure for about 2 weeks, complete recovery is the rule During the febrile period, virus is found in the blood, the cerebrospinal fluid, the urine and the nasopharyngeal secretions In the other neurologic or systemic forms, which are seen only occasionally, the disease takes on the syndrome referable to the extent and the location of lesions in the CNS or other organs, these forms are sometimes fatal Paralytic manifestations are rare The virus can be recovered from the CNS or from the lungs of patients dying of the disease There are many instances in which neutralizing antibody is found in the blood of a person who gives no history of an attack of the ailment, these are examples of clinically inapparent infection

In the experimental disease in man (Lépine et al, 1937) the incubation period was from 36 to 72 hours, which was followed by 2 or 3 febrile waves enduring over a 3-week period, the last febrile reaction was accompanied in about 50 per cent of the subjects by headache, vomiting and positive Kernig sign that lasted for 2 or 3 days

During the acute phase the blood count reveals a mild polymorphonuclear leukocytosis The cerebrospinal fluid is under increased pressure, protein is slightly increased, but the amount of sugar may be normal or abnormally low, pleocytosis occurs with usual counts of 150 to 400 lymphocytes per cu mm and sometimes as many as 1700 to 33 000 per cu mm have been reported

Pathologic Picture

The occasional fatal case of the encephalitic or myelitic type showed inflammatory changes in the meninges, the ependyma and the choroid plexuses characterized by marked infiltration with lymphocytes Otherwise, the lesions corresponded to those observed generally in the viral encephalitides In rare fatal cases of the acute systemic type of the disease, the lungs and the liver showed inflammatory reactions (Smadel et al, 1942)

Experimental Infection, Host Range

The virus is transmissible to man, albino mice, guinea pigs, monkeys, dogs, rats, gray mice, chimpanzees and chick embryos. Rabbits, pigs and birds are apparently insusceptible The laboratory animals of choice for in-

plastic disease *Its etiology remains obscure,* although certain workers (Mollaret et al, 1956) consider its causal agent to be a virus related to the psittacosis-lymphogranuloma group The syndrome was first described under the name *la maladie des griffes de chat,* Debré et al., 1950, but since only a portion of the cases have a history of contact with cats, the term "benign lymphoreticulosis," proposed by Mollaret et al, 1950, may have some justification. The disease is not uncommon

Most patients have a history of a cat-bite, a cat-scratch or merely having been licked by a cat a few days prior to illness Contact with cat excreta has resulted in a familial outbreak of the disease A cutaneous lesion resembling a small pustular furuncle appears in about 50 per cent of cases and may represent the primary pathologic reaction Following an incubation period of 2 to 6 weeks the regional lymph nodes draining the area become inflamed, swollen, painful and may suppurate, mild generalized lymphadenopathy and splenomegaly are also present. Malaise, chills and fever are common, and a macular rash over the extremities is not unusual (Greer and Keefer, 1951, Daniels and MacMurray, 1952)

In the majority of cases the illness is mild and of short duration, but suppurative lymph nodes may persist for months and require surgical drainage or removal Other variant chronic forms include a conjunctivitis resembling Parinaud's oculoglandular syndrome (Debré and Job, 1954) and meningo-encephalitis (Stevens, 1952) Included in a differential diagnosis are Hodgkins disease, lymphogranuloma venereum, tularemia, acute and chronic bacterial adenitis and other diseases involving the lymphatic system Fatal cases are rare (Debré and Job, 1954)

Successful serial passage of the agent in lymph-node suspensions to the monkey, *Aethiops sabaeus,* has been reported by Mollaret et al. (1951, 1956), but macacus monkeys and baboons were resistant. These findings were not confirmable by Debré and Job (1954), who were also unable to infect mice, guinea pigs, rabbits, ferrets, dogs, cats, embryonated eggs or tissue cultures of human [illegible] matory reticular and endothelial hyperplasia and early sclerosis at the periphery of the granuloma Germinal follicles contain small abscesses which coalesce to form larger areas of necrosis Spontaneous or surgical drainage is followed by rapid healing Diagnosis is facilitated by an intradermal and generalized febrile reaction resembling the Frei test to heat-inactivated suspensions of infected lymph nodes or pus, Mollaret et al, 1950 (Greer and Keefer, 1951). The technic of preparing the antigen is outlined by Debré and Job (1954).

The etiology of this infectious disease remains unsettled Cats harboring the virus remain well, and since they have negative intradermal reactions may perhaps be only passive carriers. Evidence that the agent of cat-scratch disease belongs to the psittacosis-lymphogranuloma group of viruses has been presented in a series of reports by Mollaret et al (1956) Inoculation of cercopithecus monkeys with infectious human material produced a granulomatous lymphadenitis which could be transmitted serially Stained sections of human and monkey nodes contain large members of intracellular and extracellular granules resembling the elementary bodies of psittacosis Although the Frei test is usually negative, sera of persons convalescent from cat-scratch disease will often fix complement in the presence of the psittacosis-lymphogranuloma group antigen On the other hand, the failure of cat-scratch material to infect embryonated eggs (Debré and Job, 1954) leaves unsettled the relationship of this virus to other agents What, if any, connection exists between the viruses of cat-scratch disease and feline pneumonitis (Baker, 1944) also remains to be determined

Because most patients recover spontaneously, the effect of treatment is difficult to appraise Chlortetracycline and other broad-spectrum antibiotics are reported to accelerate recovery

LYMPHOCYTIC CHORIOMENINGITIS

(Synonyms: LCM; choriomeningitis, *maladie d'Armstrong*)

Introduction

Lymphocytic choriomeningitis is an endemic viral infection of lower animals, es-

pecially the mouse, in which the CNS and particularly the meninges and the choroid plexuses are involved, it is transmissible to man in whom the infection produces a marked diversity of signs and symptoms

History

The virus of lymphocytic choriomeningitis (LCM) was isolated from a monkey by Armstrong and Lillie in 1934 during the course of an investigation of St Louis encephalitis Shortly thereafter this agent was found in the cerebrospinal fluid of several human patients in whom a clinical diagnosis of benign aseptic meningitis had been made (Rivers and Scott, 1935). Lepine et al (1937) demonstrated that by subcutaneous injection of the virus from mice, performed as a therapeutic measure, LCM could be induced in human beings and that the disease was transmissible in man by means of intramuscular inoculations of infected blood Natural and laboratory infections are not uncommon, and these are discussed in the general review by Janeway and Farmer (1942) The intensive investigations of Traub (1936) focused attention on rodents, particularly mice, as both a vector and a reservoir of the virus of LCM

Clinical Picture

Wallgren (1925) used the term "acute aseptic meningitis" to designate a clinical syndrome in man that he thought was a nosologic entity He described it as an acute febrile, nonfatal malady characterized by symptoms and signs of meningeal irritation and associated at times with infection of the upper respiratory tract Now it is known that acute aseptic meningitis is not a nosologic entity but represents a clinical syndrome which may be caused by more than one etiologic agent, one of which is the virus of lymphocytic choriomeningitis

Infection by this virus may assume a number of different clinical forms (Smadel et al, 1942, Adair et al, 1953) such as aseptic meningitis, grippe, meningo encephalomyelitis and acute fatal systemic disease, often it is clinically inapparent In most instances the meningeal and grippelike types prevail After an unknown period of incubation, the onset is sudden, frequently with symptoms and signs similar to those of influenza In many patients, this is all that happens, and recovery ensues promptly In others, the grippe phase is followed by definite signs of meningitis which may endure for about 2 weeks, complete recovery is the rule During the febrile period, virus is found in the blood, the cerebrospinal fluid, the urine and the nasopharyngeal secretions. In the other neurologic or systemic forms, which are seen only occasionally, the disease takes on the syndrome referable to the extent and the location of lesions in the CNS or other organs, these forms are sometimes fatal Paralytic manifestations are rare The virus can be recovered from the CNS or from the lungs of patients dying of the disease There are many instances in which neutralizing antibody is found in the blood of a person who gives no history of an attack of the ailment; these are examples of clinically inapparent infection.

In the experimental disease in man (Lépine et al, 1937) the incubation period was from 36 to 72 hours, which was followed by 2 or 3 febrile waves enduring over a 3-week period, the last febrile reaction was accompanied in about 50 per cent of the subjects by headache vomiting and positive Kernig sign that lasted for 2 or 3 days

During the acute phase the blood count reveals a mild polymorphonuclear leukocytosis The cerebrospinal fluid is under increased pressure, protein is slightly increased, but the amount of sugar may be normal or abnormally low, pleocytosis occurs with usual counts of 150 to 400 lymphocytes per cu mm and sometimes as many as 1700 to 33,000 per cu mm have been reported

Pathologic Picture

The occasional fatal case of the encephalitic or myelitic type showed inflammatory changes in the meninges, the ependyma and the choroid plexuses characterized by marked infiltration with lymphocytes Otherwise, the lesions corresponded to those observed generally in the viral encephalitides In rare fatal cases of the acute systemic type of the disease, the lungs and the liver showed inflammatory reactions (Smadel et al, 1942)

Experimental Infection, Host Range

The virus is transmissible to man, albino mice, guinea pigs, monkeys, dogs, rats, gray mice, chimpanzees and chick embryos Rabbits, pigs and birds are apparently insusceptible The laboratory animals of choice for in-

oculation of the virus are the guinea pig and the albino mouse. Mice are best infected by intracerebral and intranasal routes, infection being obtained by these routes with 10^{-7} and 10^{-3} dilutions of virus, respectively. Mice, 5 to 12 days after inoculation, develop tremors and convulsions which characteristically terminate in a few seconds in generalized rigidity; death occurs in 1 to 3 days after onset of illness. If small doses of the virus are inoculated peripherally, a nonfatal infection may be induced which immunizes the animals; large doses may cause fatal infection after intraperitoneal administration. Guinea pigs, like mice, are best infected by the intracerebral route; after subcutaneous or intraperitoneal inoculation, death may occur within 9 to 16 days. The virus can be found in the brain, blood, spleen, lungs and urine of mice and guinea pigs and, according to Shwartzman (1944), is firmly associated with their erythrocytes

The pathologic picture is one of a meningoplexal and perivascular infiltration by

in the kidney, the salivary gland and the pancreas. In mice, also, there is often serous pleurisy, serous peritonitis and hepatitis; and necrosis, hemorrhage and serofibrinous exudate are observed in the lymphatic organs. Hepatitis is seen also in infected guinea pigs and monkeys. Thus, in experimental infection, the virus is generalized through the CNS and the viscera and is associated with inflammatory lesions which persist for a long time during convalescence (Lillie, 1950). Different strains of the virus vary greatly in their pathogenicity for and behavior in experimental animals.

The virus occurs naturally in several species of animals, mice, guinea pigs, monkeys and dogs have been found to harbor it. Traub (1935) found that the spontaneous disease in mice is transmitted by mothers to their young in utero or shortly after birth and that the virus is propagated or maintained in a colony by normal-appearing carriers.

Etiology

The diameter of the virus is from 40 to 60 mμ as determined by filtration through gradocol membranes, and from 37 to 55 mμ as estimated from results of ultracentrifugation. It passes through Berkefeld V, N and W candles and Seitz filters. It is preserved in 50 per cent buffered glycerol, by being kept in the frozen state at $-70°$ C, and by lyophilization; in a brain suspension it is not stable at room temperature. It can be cultivated in vitro in minced mouse- or chick-embryo tissue suspended in a mixture of salt solution and serum, on the chorio-allantois, or in the yolk sac of 11- or 12-day-old chick embryos. The membranes and the brains yield virus with a titer of up to 10^{-7} by intracerebral titration in mice. However, infected embryos hatch, and the chicks survive. Smadel and Wall (1941) have demonstrated the presence of a specific soluble substance separable from the active virus in the organs, chiefly spleens, of infected guinea pigs and mice.

Complement-fixing and neutralizing antibodies appear in the serum of convalescent human beings; the former is first noted from about 1 to 3 weeks after onset of illness, the latter not until 6 to 10 weeks. Neutralizing antibody is known to persist for at least 3 years in persons who have had the disease, while the amount of complement-fixing antibody begins to decline 3 to 6 weeks after the onset of an attack Both kinds of antibody are also produced in experimental animals after infection with active virus or after immunization with inactive virus, both appear within 10 days; how long they endure is not known.

Diagnosis

Since the clinical course of lymphocytic choriomeningitis cannot be differentiated from that which follows infection with a number of other neurotropic viruses, including poliomyelitis, ECHO virus infection, mumps, herpes, etc, definitive diagnosis rests upon isolation and identification of the agent and serologic tests. The virus may be obtained from the blood, the cerebrospinal fluid or CNS tissues. The mouse and the guinea pig are the species of choice for isolation studies, but one should be certain that the stock of animals used for diagnostic purposes is free of virus Neutralization and complement-fixation tests are performed with acute and convalescent

sera Detailed diagnostic procedures can be found in Adair et al (1953) and in Hammon (1956).

Treatment

There is no specific treatment. Antifolic acid compounds were capable of prolonging or sparing the lives of experimentally infected mice, although their prolonged viremia was unaffected (Haas and Stewart, 1956)

Epidemiology

The disease occurs in persons of all ages, with 20 to 30 years the most frequent and males and females being equally affected Most cases occur in winter and spring Its incidence in the United States is difficult to estimate Adair et al. (1953) found LCM to be the cause in approximately 9 per cent of 854 sporadic cases of aseptic meningitis However, neutralizing antibody was present in 11 per cent of 2000 sera collected at random from persons without a history of clinical LCM (Armstrong, 1941). Moreover, the disease assumes an important role in public health because its virus is present in high titer in certain lower animals, such as the gray or house mouse (*Mus musculus*) The virus may persist in carrier mice throughout their lives and exist as an epizootic infection in a colony (Traub, 1936). Rowe (1954) has demonstrated that acquired immunity in the albino mouse is associated with persistence of the virus The virus escapes from the mouse by way of the nasal secretions, the urine, the feces and the semen, thus possibly contaminating the habitat of man It has been suggested by Armstrong (1941) that dust may be a source of human infection

The virus most likely enters man by the upper respiratory tract, laboratory infections are not uncommon Shaughnessy and Milzer (1939) have suggested that arthropods, such as culicine mosquitoes, stable flies, wood ticks and baby lice, may serve as vectors for transmitting the disease from infected to normal rodents and to man Still another possible means of conveying infection from mouse to mouse, and mouse to man has been suggested by Syverton et al (1947), namely, through superinfected *Trichinella spiralis* or its larvae

Control Measures

The eradication of rodent or other animal carriers, together with the maintenance of high standards of sanitation, would appear to be important measures for control of the disease in a population. A patient's excretions should be disinfected, since they may contain the virus

ENCEPHALOMYOCARDITIS

(Synonyms: Columbia-SK, MM, Mengo, F viruses, etc. See Table 41)

Introduction

Members of this group of viruses were discovered in widely separated areas and in certain instances were regarded as different agents. However, they are now generally recognized as strains of a single virus which are immunologically indistinguishable All are highly infectious for several rodents, which probably serve as reservoirs. Encephalomyocarditis infection in man clinically resembles other neurotropic viral diseases

History

The first of these strains to be recognized was isolated by Jungeblut and Sanders, 1940, from cotton rats which were being used for the passage of the Yale-SK strain of polio-

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from hamster brain inoculated with material from human spinal cord and medulla taken from a patient who had died of clinically diagnosed paralytic disease, Jungeblut and Dalldorf, 1943 Because both agents were discovered under circumstances where material, either known to be or suspected of being infected with poliomyelitis virus, was being passaged through rodents, considerable confusion surrounded the relationship of the agents to poliomyelitis virus. Indeed, for a time it was thought that they represented murine variants of the latter. Subsequent study has failed to provide adequate evidence of any antigenic relationship between the agents of this group and poliomyelitis

The encephalomyocarditis (EMC) and Mengo encephalomyelitis strains were isolated from captive monkeys in Florida, Helwig and Schmidt, 1945, and in Uganda, Dick et al, 1948, respectively. Strains of Mengo virus were also obtained from mosquitoes (*Taeniorhynchus fuscopennatus*) and a wild mongoose caught in the vicinity of a monkey compound at Entebbe, Uganda

Subsequent isolations of EMC virus have

TABLE 41. ISOLATIONS OF STRAINS OF ENCEPHALOMYOCARDITIS VIRUS FROM MAN AND ANIMALS

MAN

Date	Clinical Signs	Material	Test Animal	Strain Designation	Authors	Location	Remarks
1943	Fatal paralysis	CNS	Hamster	MM	Jungeblut and Dalldorf	New York	
1947	Encephalitis	Serum	Mouse	Mengo	Dick et al	Uganda	Possible laboratory infection
1949	Paralytic Poliomelitis	Stool	Mouse Monkey	AK	Verlinde et al	Netherlands	From a child
1949	Guillain-Barré Syndrome	CSF	Mouse	*Lz 32*	*Beller* and Keller	*Germany*	*Isolation made* in 1943
1950	Aseptic Meningitis	Blood CSF	Mouse	F	Beiling and Koch	Germany	One of four similar cases
1952	Myelitis Encephalitis	Stool	Mouse	Ortheb	Vivell and Mauer	Germany	From a child
1953	Poliomyelitis	Stool	Mouse	S V W	Verlinde et al	Netherlands	From 3 children

ANIMAL

Date	Species	Material	Test Animal	Strain Designation	Authors	Location	Remarks
1940	Cotton Rat	CNS	Mouse	Columbia-SK	Jungeblut and Sanders	New York	
1945	Chimpanzee	Pleural Fluid	Mouse	EMC	Helwig and Schmidt	Florida	Animal died with signs of cardiac failure
1948	Mongoose and Monkey	Serum CNS	Mouse	Mengo	Dick et al	Uganda	
1952	Baboon	Viscera	Mouse	—	Kissling et al	Florida	From an animal farm
1953	Monkey	CNS	Mouse	—	Kissling et al	Florida	From an animal farm
1954	Squirrel	CNS	Mouse	—	Kissling et al	Florida	Squirrel appeared rabid

been made in the southeastern United States from both primates and rodents These are summarized in Table 41

Human infections with encephalomyocarditis have been associated with a variety of clinical signs varying from a mild febrile illness to a severe encephalomyelitis. In several instances close proximity to rodents has been noted. Beller and Keller (1949) isolated a strain in 1943 from the cerebrospinal fluid of a child with Guillain-Barre type of polyradiculitis The Mengo strain was isolated from a laboratory worker who developed a severe meningo-encephalitis while engaged in studies on this virus, Dick et al, 1948 Additional isolates have been reported from adults and children with nonfatal illnesses diagnosed as paralytic poliomyelitis, aseptic meningitis and encephalomyelitis (See Table 41.) There is some evidence that EMC infection may appear in localized outbreaks (Bieling and Koch, 1952). A mild febrile disease, called "3-day fever," appeared in epidemic form among U. S. troops quartered in Manila in *1945-46* *Neutralizing antibodies* to EMC were demonstrated in the sera taken from many of these patients during convalescence (Smadel and Warren, 1947)

CLINICAL PICTURE

The clinical features are varied in those few cases in which the evidence that they were

caused by EMC virus is acceptable A febrile CNS disease with a lymphocytic pleocytosis sometimes accompanied by one or more signs of paralysis was the most common finding in 14 well-studied cases (Gajdusek, 1955) Myocarditis has not been one of the symptoms of EMC infections in humans

The single known human infection with the Mengo strain was characterized by an elevated temperature lasting 4 days accompanied by severe headache, nuchal rigidity, photophobia, vomiting and short episodes of delirium, Dick et al, 1948 The patient recovered rapidly, the only sequelae being a transient unilateral nerve deafness Mengo virus was isolated from the blood on the 1st and the 2nd days of illness by animal inoculation, and neutralizing antibodies were demonstrated in the patient's serum following recovery.

In the cases occurring in the Philippines, onset of the disease was sudden, with severe headache and a moderately high fever, reaching 104° F in some instances The fever lasted for 2 or 3 days and was often accompanied by pharyngitis, stiff neck, positive Kernig's sign and hyperactive deep reflexes An occasional patient was comatose on admission to the hospital The only notable laboratory finding was pleocytosis of from 50 to 500 cells, principally lymphocytes in the spinal fluid All patients recovered promptly and usually were discharged from the hospital in 4 or 5 days There were no known sequelae, and no signs of cardiac disease were observed On the basis of these findings, a clinical differentiation of encephalomyocarditis from aseptic meningitis, nonparalytic poliomyelitis and the denguelike fevers was not possible

Pathologic Picture

Nothing is known of the pathologic picture in human beings, because no deaths have been reported among the patients with proved virus infection

Experimental Infection, Host Range

Although all strains of the encephalomyocarditis group can multiply in most laboratory animals, the response varies with the host Mice and hamsters of all ages die following inoculation by any of the usual routes with a $10^{-7.0}$ dilution of a suspension of brain tissue from moribund mice, hamsters or cotton rats Within 72 or 96 hours, such an inoculation produces signs consisting of ruffled fur, lethargy and flaccid paralysis, which are invariably followed by prostration and death When greater concentrations of virus are given intracerebrally, death occurs as early as 18 hours after inoculation; animals receiving this amount of virus show no paralysis but die with signs of an acute encephalitis Occasionally, mice can be infected with virus-contaminated drinking water Guinea pigs and rabbits experience an infection characterized by fever for the first 4 or 5 days after inoculation Paralysis, occasionally fatal, occurs in 10 to 50 per cent of infected guinea pigs, all animals develop infection as evidenced by the appearance of neutralizing antibody The disease is not fatal in rabbits The resistance of the albino rat increases with age (Kilham et al, 1955) and the adult rat undergoes only an inapparent infection following a massive inoculation, although considerable virus may persist in the central nervous system for several weeks The response of rhesus monkeys to viruses of the EMC group is variable, ranging from an acute febrile course followed by death within the first week to a mild infection characterized by a short fever and subsequent appearance of serum antibodies Paralysis in monkeys is not infrequent, but the animals usually recover The viruses can be propagated in chick embryos, causing death in 72 to 96 hours without pathognomonic lesions Viremia occurs during some portion of the febrile period in infected animals of all species mentioned above Virus usually persists for several days, and blood specimens when titered in mice are infectious in dilutions of $10^{-3.0}$ to $10^{-4.0}$ Viruses of the EMC group can be cultivated in Ehrlich ascites cells, the L strain of mouse fibroblasts, the HeLa and the KB Lines of human malignant cells Strains differ in their cytopathogenicity with the EMC and the Mengo strains being of greater pathogenicity (Jungeblut, 1958)

Lesions in experimentally infected animals are found in the entire central nervous system and in striated and cardiac muscles This is a common feature of the agents of the

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1953	Monkey	CNS	Mouse	—	Kissling et al	Florida	From an animal farm
1954	Squirrel	CNS	Mouse	—	Kissling et al	Florida	Squirrel appeared rabid

been made in the southeastern United States from both primates and rodents These are summarized in Table 41.

Human infections with encephalomyocarditis have been associated with a variety of *clinical signs varying from a mild febrile illness* to a severe encephalomyelitis In several instances close proximity to rodents has been noted. Beller and Keller (1949) isolated a strain in 1943 from the cerebrospinal fluid of a child with Guillain-Barré type of polyradiculitis The Mengo strain was isolated from a laboratory worker who developed a severe meningo-encephalitis while engaged in studies on this virus, Dick et al, 1948 Additional isolates have been reported from adults and children with nonfatal illnesses diagnosed as paralytic poliomyelitis, aseptic meningitis and encephalomyelitis (See Table 41.) There is some evidence that EMC infection may appear in localized outbreaks *(Bieling and Koch, 1952)*. A mild febrile disease, called "3-day fever," appeared in epidemic form among U S troops quartered in Manila in 1945-46 Neutralizing antibodies to EMC were demonstrated in the sera taken from many of these patients during convalescence (Smadel and Warren, 1947)

CLINICAL PICTURE

The clinical features are varied in those few cases in which the evidence that they were

caused by EMC virus is acceptable A febrile CNS disease with a lymphocytic pleocytosis sometimes accompanied by one or more signs of paralysis was the most common finding in 14 well-studied cases (Gajdusek, 1955) Myocarditis has not been one of the symptoms of EMC infections in humans

The single known human infection with the Mengo strain was characterized by an elevated temperature lasting 4 days accompanied by severe headache, nuchal rigidity, photophobia, vomiting and short episodes of delirium, Dick et al, 1948 The patient recovered rapidly, the only sequelae being a transient unilateral nerve deafness Mengo virus was isolated from the blood on the 1st and the 2nd days of illness by animal inoculation, and neutralizing antibodies were demonstrated in the patient's serum following recovery.

In the cases occurring in the Philippines, onset of the disease was sudden, with severe headache and a moderately high fever, reaching 104° F in some instances The fever lasted for 2 or 3 days and was often accompanied by pharyngitis, stiff neck, positive Kernig's sign and hyperactive deep reflexes An occasional patient was comatose on admission to the hospital The only notable laboratory finding was pleocytosis of from 50 to 500 cells principally lymphocytes, in the spinal fluid All patients recovered promptly and usually were discharged from the hospital in 4 or 5 days There were no known sequelae, and no signs of cardiac disease were observed On the basis of these findings, a clinical differentiation of encephalomyocarditis from aseptic meningitis, nonparalytic poliomyelitis and the denguelike fevers was not possible

Pathologic Picture

Nothing is known of the pathologic picture in human beings, because no deaths have been reported among the patients with proved virus infection

Experimental Infection, Host Range

Although all strains of the encephalomyocarditis group can multiply in most laboratory animals, the response varies with the host Mice and hamsters of all ages die following inoculation by any of the usual routes with a $10^{-7.0}$ dilution of a suspension of brain tissue from moribund mice, hamsters or cotton rats Within 72 or 96 hours, such an inoculation produces signs consisting of ruffled fur, lethargy and flaccid paralysis, which are invariably followed by prostration and death When greater concentrations of virus are given intracerebrally, death occurs as early as 18 hours after inoculation; animals receiving this amount of virus show no paralysis but die with signs of an acute encephalitis Occasionally, mice can be infected with virus-contaminated drinking water Guinea pigs and rabbits experience an infection characterized by fever for the first 4 or 5 days after inoculation Paralysis, occasionally fatal, occurs in 10 to 50 per cent of infected guinea pigs; all animals develop infection as evidenced by the appearance of neutralizing antibody The disease is not fatal in rabbits The resistance of the albino rat increases with age (Kilham et al, 1955) and the adult rat undergoes only an inapparent infection following a massive inoculation, although considerable virus may persist in the central nervous system for several weeks The response of rhesus monkeys to viruses of the EMC group is variable, ranging from an acute febrile course followed by death within the first week to a mild infection characterized by a short fever and subsequent appearance of serum antibodies Paralysis in monkeys is not infrequent, but the animals usually recover The viruses can be propagated in chick embryos, causing death in 72 to 96 hours without pathognomonic lesions Viremia occurs during some portion of the febrile period in infected animals of all species mentioned above Virus usually persists for several days, and blood specimens when titered in mice are infectious in dilutions of $10^{-3.0}$ to $10^{-4.0}$ Viruses of the EMC group can be cultivated in Ehrlich ascites cells, the L strain of mouse fibroblasts, the HeLa and the KB Lines of human malignant cells Strains differ in their cytopathogenicity with the EMC and the Mengo strains being of greater pathogenicity (Jungeblut, 1958)

Lesions in experimentally infected animals are found in the entire central nervous system and in striated and cardiac muscles This is a common feature of the agents of the

EMC group, hence their generally accepted designation The extent of the pathologic changes is related to the portal of inoculation and duration of disease. If the virus is inoculated directly into a muscle, e.g., the gastrocnemius, there is a rapidly progressing necrosis of the muscle fibers accompanied by edema and inflammatory reaction. Cardiac lesions require longer to develop, and, in animals dying after a number of days, these appear grossly as pale yellow plaques 0.5 to 2.0 [illegible]
tent [illegible]
a si [illegible]
myocarditis affecting a large area of the heart. The pathologic process appears to begin with a mild perivascular monocytic infiltration and with swelling and loss of striations of muscle fibers which progress to complete necrosis. Cellular infiltrations are usually extensive, lymphocytes and histiocytes predominating (Schmidt, 1948) Focal granulomatous lesions containing multinucleated giant cells have also been observed occasionally in the hearts of monkeys and mice. The viruses of the EMC group are also highly neurotropic with the severity of lesions varying with the duration of the disease Thus, in animals which survive only 1 or 2 days lesions in the CNS are minimal. When, however, the incubation period is prolonged to 4 or 5 days by giving a small dose of virus by a peripheral route, the usual lesions of encephalitis are encountered, which consist of congestion of the capillaries and infiltration of lymphocytes and mononuclear cells into the subarachnoid space, perivascular spaces and cerebral parenchyma However, neither the meningitis nor the cuffing is intense. The most striking and characteristic change consists of sharply demarcated focal areas of necrosis scattered throughout the central nervous system, most common in the cerebellum where they usually involve the granular and Purkinje cells layers.

Etiology

The Columbia-SK, MM, EMC and Mengo encephalomyelitis agents are small viruses, readily passing through Berkefeld and Seitz filters and through gradocol membranes with average pore-diameters of 30 mμ; thus, according to results with gradocol membranes, they have diameters of 8 to 15 mμ. However, direct visualization by electronmicroscopy or determination of the sedimentation constants of highly purified preparations indicates a particle size of 25 to 29 mμ (Col.-SK, Jungeblut and Bourdillion, 1943; EMC, Weil et al., 1951). They are quite stable when stored at −70° C. and can also be preserved in 50 per cent glycerol or 0.05 molar glycine. They are inactivated by a temperature of 60° C. for 30 minutes and usually lose their infectivity following lyophilization Specific antibodies which cross-react equally with all strains appear in the sera of convalescent animals and man. In the rhesus monkey and in man antibodies may be present late in the first week or early in the second. Tests for neutralizing antibodies are performed in mice by means of intracerebral or intraperitoneal inoculation of serum-virus mixtures Complement-fixing antibodies have been found in the sera of immune hamsters, guinea pigs and rats (Warren et al., 1949b). Ability of members of the EMC group to agglutinate sheep erythrocytes was first reported by Hallauer (1949, 1951) and later by Olitsky and Yager (1949) and Gard and Heller (1951) Agglutination occurs at 4° C., and virus is eluted from the cells above 20° C. Agglutination is inhibited by specific immune serum The Columbia-SK, MM, EMC and Mengo strains of virus are indistinguishable by cross-protection, neutralization, complement-fixation and hemagglutination-inhibition technics. Differences in pathogenicity between the strains do not provide adequate criteria for their differentiation, as variations in strain pathogenicity for a given host or route of inoculation are readily produced by repeated adaptive passes An achievement of fundamental importance was made by Colter et al. (1957), who were able to isolate an infective ribonucleic acid from Ehrlich ascites cells infected with the Mengo strain, the first such preparation from a virus infecting mammalian cells.

Diagnosis

The diagnosis of encephalomyocarditis in man rests on the isolation and the identification of the virus and the demonstration of the development during convalescence of specific antibodies. Because of the possibility that laboratory animals, especially rodents, may harbor the virus, multiple isolations are desirable for a definitive diagnosis. The important differential generic characteristics that

serve to distinguish these strains from other known viruses are:

1. Small size and the ability to hemagglutinate; a property also shared by the ECHO viruses

2. A broad host range including the embryonated egg.

3. High infectivity by peripheral routes in a wide variety of adult hosts and disease in these hosts.

The symptoms and signs of the disease in man are not sufficiently diagnostic to permit its differentiation from a number of febrile neurologic infections

Treatment

Treatment is symptomatic

Epidemiology

Primary isolation of strains of EMC virus have been reported from a variety of rodents, the rhesus monkey, the chimpanzee and the mandrill baboon (Kissling et al, 1956) In addition, several species of Taeniorrhynchus mosquitoes were found naturally infected in Uganda, Dick et al, 1948 (Horgan, 1951) There is considerable evidence that rodents, in particular certain species of wild rats, constitute a reservoir for EMC virus. The albino rat, after intracerebral inoculation of virus, undergoes an inapparent infection, and considerable virus persists for several weeks in the central nervous system and the [illegible]

[illegible]

sues, urine and feces (Kilham et al, 1956) Contact transmission within cotton rats and infection by feeding in the case of albino mice has been demonstrated Further evidence that rats in nature may harbor strains of EMC virus is the finding of neutralizing antibodies in a significant proportion of *Rattus norvegicus* and *R alexandrinus* collected in certain areas of the United States and Canada (Warren et al, 1949a) and *R kyjabius* and *R coucha* trapped in Uganda (Horgan, 1951). Tests for antibody in other small animals in the same areas have been uniformly negative

Surveys of the incidence of neutraliz[illegible]

[illegible] 1954, Kellel and Vivell, 1954). These have shown that, whereas less than 3 per cent of healthy individuals have EMC antibody, a higher percentage (12% to 16%) of sera from cases of CNS disease neutralize this virus

Control Measures

No control measures are known

WARTS: VERRUCAE

There are several types of wart which vary in appearance, location and preference of age (van Rooyen and Rhodes, 1940). Those usually recognized are common warts, digitate warts, juvenile (plane) warts, filiform warts, genital warts and laryngeal papillomata. Common warts usually occur as discrete, oval, gray, dry growths on the hands Juvenile warts are small, flat growths usually occurring in groups on the face Digitate warts are growths broken into folds and are found on the face and the scalp. Filiform warts are delicate threadlike growths on the eyelids and the neck. Genital warts are small, gray, rough nodules which appear in the coronal sulcus of the penis or on the labia and around the vaginal orifice During pregnancy they increase in size but become smaller after its termination In the female, because of the moisture around the genital regions, warts may become large and secondarily infected with bacteria Laryngeal papillomata are flat or pedunculated and occur singly or in groups, they may recur after removal

It has been definitely shown that an agent filterable through Berkefeld candles of all grades of porosity is responsible for warts. The virus survives a temperature of 50° C for 30 minutes Furthermore, most workers believe that a single etiologic agent is responsible for the different types of wart mentioned Examination of extracts of certain papillomata of the skin by means of the electronmicroscope have revealed virus-like particles which tended to aggregate in [illegible]

[illegible] Kingery, 1921) Laryngeal papillomata are the only warts that have been transmitted to lower animals, and this was accomplished by injecting the infectious material into the

vaginal mucosa of bitches. Warts of dogs and cattle are not transmissible to man The incubation period in the experimental disease has been as follows in reported experiments. 1, 6, 8 and 20 months, respectively

If warts are made to bleed, new lesions may occur in neighboring parts of the skin. They are spread by direct or indirect contact in barber shops, bathing pools, chiropodists' offices, hairdressing establishments and other places of similar character The epithelium is the only tissue involved by some warts and shows a hyperkeratosis and many mitotic figures In some warts the deeper tissues are involved and at times have an inflammatory reaction As a rule, no specific inclusions have been found Warts usually disappear spontaneously, and there is evidence that some immunity is present in a patient after recovery Many kinds of treatment have been devised for warts, but none is specific

MOLLUSCUM CONTAGIOSUM

Molluscum contagiosum has been known as a clinical entity since 1817 The incubation period as determined by inoculation of human volunteers has been stated by 3 groups of investigators to be 50, 14 to 25, and 35 days, respectively The disease is characterized by the formation of multiple discrete nodules, limited to the epidermal layer of the skin, with an average diameter of 2 mm. The lesions may appear on the face, the arms, the legs, the buttocks, the genitalia, or the scalp, rarely, in the mucous membranes of the mouth, never on the soles and the palms The nodules are usually pearly white and painless, and at the top of each there is often an opening through which a small, white core can be seen Some of the lesions may become secondarily infected with bacteria and break down The disease is chronic, and several months may be required for recovery.

Lesions involve circumscribed areas of the epidermis which become thickened by hyperplasia and hypertrophy of infected epithelial cells. In the germinal layer, hyperplasia is evidenced by an increase in mitotic figures. In cells above the germinal layer, definite pathologic changes in the nuclei and the cytoplasm become obvious As the surface of the epidermis is approached, these changes become more and more marked so that eventually each cell is many times larger than normal, and the cytoplasm is filled with a large, hyaline, acidophilic, granular mass known as the molluscum body, which pushes the nucleus to the edge of the cell. A fully developed lesion is usually loculated, and there is very little reaction in the corium unless a nodule is secondarily infected by bacteria

The disease is not a serious one but is of particular interest because of discussions concerning the architecture and the significance of the molluscum body which at one time was considered a protozoan parasite. According to Goodpasture and King, 1927, and Goodpasture and Woodruff, 1931, the molluscum body is surrounded by desiccated cytoplasmic protein which extends into the structure to form trabeculae The covering and the trabeculae can be digested away by trypsin, this procedure leaves a sticky, gelatinous mass, within which numerous elementary bodies, first described by Lipschutz, 1907, are embedded According to van Rooyen (1938, 1939), the molluscum body is not a mixture of cytoplasmic material and virus elementary bodies but constitutes a foreign entity in the cell resulting from the growth of an elementary body which passes through developmental stages finally to form a large structure surrounded by a membrane with an operculum at one pole and filled with elementary bodies He believes that this structure has certain resemblances to the sporangium of a fungus, for example, *Rhino-sporidium seeberi*, which causes polyps in the nasal mucous membranes. Thin sections of molluscum contagiosum lesions have been studied by means of the electronmicroscope and histochemical technics (Rake and Blank, 1950; Banfield et al, 1951) In the early infected cell, elementary bodies are scattered in a random fashion throughout the cytoplasm These later become aggregated and surrounded by a large network of spongy matrix In the larger inclusion bodies this matrix is divided into cavities in which are clustered numerous elementary bodies. Within the elementary bodies are still smaller particles, with diameters of 80 to 100 mμ, which may represent some simpler structural unit. Molluscum bodies have diameters ranging from 20 to 30 μ, Lipschutz, 1907, while an elementary body

is brick-shaped and has average diameters of 200×300 mμ (Strauss et al, 1949) The latter stains well by Morosow's method Elementary bodies are said by various workers (van Rooyen and Rhodes, 1940) to pass through Chamberland L_1 and Berkefeld V filters The virus retains its activity in 50 per cent glycerol for at least a month A soluble, heat labile complement-fixing antigen has been prepared from suspensions of molluscum nodules by Mitchell (1953) However, only a fraction of cases possess complement-fixing antibodies and this procedure is not of diagnostic value No serologic relationship was demonstrable between molluscum virus and vaccinia, or fowlpox viruses

Man is the only known host, attempts to infect monkeys, apes, sheep, fowl, rabbits, guinea pigs, mice and embryonated eggs have been unsuccessful Molluscum contagiosum has a world-wide distribution, is particularly prevalent in certain areas, for example, Edinburgh; it is seen most frequently in children, but persons of all ages may be attacked, it is transmitted by personal contact or by fomites There is no specific treatment Some workers have stated that x-rays lead to rapid healing of the lesions, others have noted that healing takes place following bacterial invasion

EPIDEMIC VIRAL GASTROENTERITIS

(SYNONYMS Acute infectious gastroenteritis winter vomiting disease, febrile nonbacterial gastroenteritis)

Dysfunction of the human alimentary tract characterized by diarrhea and mild systemic signs may be associated with infection by a number of bacterial and viral agents The latter include poliomyelitis, certain of the ECHO viruses, Coxsackie A and B viruses, infectious hepatitis and herpes virus With the possible exception of certain of the ECHO types (Chap 26) these viruses are infrequently found in uncomplicated forms of epidemic gastroenteritis On the other hand, several agents of comparative obscurity have been isolated from outbreaks of this illness, and these are described in this section

The epidemic viral gastroenteritides are a nonseasonal, widespread communicable family of diseases whose clinical and epidemiologic features as they occur in the United States and abroad have been well delineated (Cook and Marmion, 1947, Gordon et al, 1949, Reimann et al, 1945, Jordan et al, 1953) Although its frequent appearance in public institutions and small communities has stimulated the majority of reports, cases may occur sporadically or in small endemic foci It affects infants as well as adults of all ages with some or all of the following symptoms anorexia, vomiting, gastric pain, fever, dizziness, chills, malaise and generalized muscular aches Diarrhea, usually of a watery type, may or may not be a cardinal symptom. Thus in an epidemic some individuals may have persistent vomiting without diarrhea while others suffer an almost intractable fecal incontinence Appearance of the vomitus or the stools is not remarkable and may differ from one outbreak to another Extremely high temperatures, respiratory or CNS signs are not usually observed Although onset is often very acute, the illness is of short duration, rarely lasting longer than 2 or 3 days, and, like its onset recovery is usually fast Fatalities are rare and confined to aged persons

Since repeated bacteriologic studies of these outbreaks have been fruitless, a viral cause of this syndrome has long been postulated, and this is supported by the isolation of several filterable agents from local epidemics Gordon et al (1949, 1956) found that volunteers who ingested filtrates of stool from patients with epidemic gastroenteritis rapidly developed severe watery diarrhea but little or no elevation of temperature The agent, designated Marcy, could be serially transmitted in human beings but has failed to infect human, rhesus or avian cells in tissue culture or suckling mice Human reinfection experiments indicated that an attack conferred immunity for at least 15 months A virus similar to the Marcy agent has been also found in Japan (Kojima et al, 1948) Another enteric virus, FS, capable of inducing a diarrhea but one in which fever is also a prominent symptom, was investigated by Jordan et al (1953), who demonstrated differences between the Marcy and the FS agents on the basis of their incubation period, clinical syndrome and the absence of cross-immunity Although the rapid spread of epidemic gastroenteritis suggests the respiratory tract as a portal of entry, attempts to transmit the

FS and the Marcy agents by the inhalation of infective throat washings have been unsuccessful. It is not unlikely that further study will disclose additional antigenic types of enteric viruses which are capable of causing transient gastroenteritis and fail to propagate in vitro.

A diagnosis of epidemic viral gastroenteritis is made on the basis of a clinical picture unlike that of bacterial gastroenteritis or dysentery, its infectiousness and rapid spread within a focus, and on negative bacteriologic findings. There is no specific therapy. Since it appears that entrance of the virus into the mouth results in infection, control measures involve strict attention to isolation and the general sanitary measures applicable to highly contagious diseases.

It is unknown whether or not epidemic viral gastroenteritis is related to the more severe epidemic diarrhea of the newborn in which pathogenic coliform bacteria are not involved. It is noteworthy that in this disease adult infection is rare, the course is more prolonged, and fatal cases are not uncommon. Search for an enterotropic virus in such outbreaks has yielded 2 agents. Light and Hodes (1943) produced a transmissible, bloody, mucoid diarrhea in calves by the intranasal administration of stool filtrates from sick infants Recovered calves could not be reinfected, nor were the stools of normal infants or calves able to produce the disease (The latter fact may be of significance in differentiating this virus from that described by Baker [1943] as causing diarrhea in calves.) Buddingh and Dodd (1944) investigated the etiology of nonfatal sporadic stomatitis and diarrhea of infants. A filterable virus was detected in throat-washings and stools which was capable of causing an iritis and conjunctivitis in the eye of the rabbit. It does not appear to be herpes simplex virus on the basis of the nonkeratitic nature and the short duration of the ocular lesions in rabbits, and

evidence that adults in close contact with the disease may become asymptomatic temporary carriers The relation of this and the other stool isolates described above to the better characterized enteroviruses remains obscure.

GENERALIZED SALIVARY GLAND VIRUS INFECTION

(SYNONYMS: Cytomegalic inclusion disease, submaxillary gland virus)

A virus disease of man and animals characterized by the presence of intracytoplasmic and intranuclear inclusion bodies in the cells of several organs, in many instances unassociated with clinical findings, was described over 50 years ago. Strains of the virus are species-specific, although the similarity of the morphologic changes they produce suggests that they are biologically closely related.

HISTORY

The first description of the inclusions typical of this disease in man was made by Jesionek and Kiolemenoglou (1904), and successful transmission using the guinea pig virus was reported in 1926 by Cole and Kuttner. The occurrence of salivary gland inclusions was subsequently observed in other species of rodents (Thompson, 1932) and monkeys (Cowdry and Scott, 1935). Smith successfully propagated salivary gland virus (SVG) from mice (1954) and from man (1956). Independently, Rowe et al. (1956) and Weller et al. (1957) recovered strains of SGV from human adenoids and liver biopsies, respectively, and the latter authors demonstrated its presence in the urine of in-

antigenically related to human strains.

CLINICAL PICTURE

The anatomic and clinical manifestations of SGV infection are varied In young infants the disease appears as a generalized blood dyscrasia associated with hepatomegaly and hepatic damage. Cerebral calcification and chorioretinitis have been reported (Weller et al, 1957). The disease is generally fatal at this age. When infection occurs in older children the clinical picture varies, depending on the organs involved. Severe diarrhea, hepatic and renal damage, pneumonia and cerebral lesions are commonly observed. It is frequently secondary to another illness and has been found with pertussis and fibrocystic disease of the pancreas (Smith and Vellios, 1950). Although diagnosed cases in adults have been rare, it appears probable that sub-

clinical infections are not infrequent The finding of virus in adenoidal tissue and in the urine of healthy persons, together with a high incidence of complement-fixing antibodies in adults (Rowe et al, 1956), suggests that SGV is a more widely distributed agent than was indicated by the number of diagnosed cases

Pathologic Picture

All strains of SGV virus, whether of rodent, simian or human origin, produce the same cytologic changes. The cells, usually in epithelial tissues, become greatly enlarged, and their nuclei contain prominent acidophilic or amphophilic bodies surrounded by a clear halo which [redacted] bounded by a sharply defined nuclear membrane. The cytoplasm may contain several small basophilic satellite bodies arranged around the periphery of the cell In fatal cases the lesions are most commonly found in the liver, the bile ducts, the renal tubules and the bronchial epithelium They are less frequent in the adrenals, the gastro-intestinal epithelium, the pancreas, the thyroid or the parathyroid. In experimentally infected guinea pigs, inclusion bodies are found only in mesodermal tissues Excellent colored photographs of SGV inclusions appear in an article by Cappel and McFarlane (1947)

Experimental Infection, Host Range

Experimental transmission of SGV disease has been obtained in mice, guinea pigs and rats, and the cultivation of rodent, simian and human strains is possible in tissue cultures of cells of the homologous species. In rodents infection is possible by a variety of routes of inoculation, although symptomatic disease does not always occur This appears to be due to widespread inapparent infection and resulting immunity, for intraperitoneal inoculation of SGV into young mice causes generalized disease in which there [redacted] severe visceral necrosis resulting in death in from 4 to 7 days (McCordock and Smith, 1936) Fatal meningitis in young rodents has been produced by intracerebral inoculation and in the case of guinea pigs by intrafetal injection of virus (Markham and Hudson, 1936).

Strains of SGV exhibit the same species specificity for tissue culture as for the host animal Human type SGV in tissue culture is neutralized by serum from infants with cytomegalic inclusion disease and occasionally by that of their mothers. Antibodies fixing complement in the presence of infected tissue culture fluid were found in a large proportion of adult human sera by Rowe et al (1956). These authors suggest that SGV may persist in human lymphatic tissue for many years.

The murine, guinea pig and human strains of SGV are destroyed by heating to 56° C for from 10 to 20 minutes, by exposure to 20 per cent ether for 2 hours, and all strains are inactive when kept at a pH below 5 0 (Hartley et al, 1957) Although SGV slowly loses infectivity at temperatures as low as −70° C., the murine strains appear to have been preserved for years in 50 per cent glycerol-saline

Diagnosis

Several methods are available for the diagnosis of this infection. Detection of typical inclusions in cells obtained by biopsy or in urinary sediments during life or in postmortem material is not difficult (Fetterman, 1952; Birdsong et al., 1956) Isolation of the virus from throat swabs, urine or tissue is best made in tissue cultures of human cells, but it should be borne in mind that fresh isolates usually grow slowly and may require careful examination over long incubation periods. The complement-fixation test employing antigens prepared from infected tissue culture has been useful as a diagnostic and epidemiologic tool (Rowe et al, 1956). These authors stress the high correlation between the isolation of SGV from children and the presence of complement-fixing antibody in their sera Neutralization tests can also be used for identification of the agent or the detection of specific antibody (Smith, 1956, Weller et al., 1957)

There is no known therapy for SGV infections

INCLUSION DISEASES OF POSSIBLE VIRUS ETIOLOGY

There are a number of human diseases wherein inclusion bodies are found after postmortem study which have been considered to be caused by one or more viruses.

Chronic, progressive encephalitis characterized by the presence of intranuclear and some-

FS and the Marcy agents by the inhalation of infective throat washings have been unsuccessful. It is not unlikely that further study will disclose additional antigenic types of enteric viruses which are capable of causing transient gastroenteritis and fail to propagate in vitro.

A diagnosis of epidemic viral gastroenteritis is made on the basis of a clinical picture unlike that of bacterial gastroenteritis or dysentery, its infectiousness and rapid spread within a focus, and on negative bacteriologic findings. There is no specific therapy. Since it appears that entrance of the virus into the mouth results in infection, control measures involve strict attention to isolation and the general sanitary measures applicable to highly contagious diseases

It is unknown whether or not epidemic viral gastroenteritis is related to the more severe epidemic diarrhea of the newborn in which pathogenic coliform bacteria are not involved It is noteworthy that in this disease adult infection is rare, the course is more prolonged, and fatal cases are not uncommon Search for an enterotropic virus in such outbreaks has yielded 2 agents. Light and Hodes (1943) produced a transmissible, bloody, mucoid diarrhea in calves by the intranasal administration of stool filtrates from sick infants Recovered calves could not be reinfected, nor were the stools of normal infants or calves able to produce the disease (The latter fact may be of significance in differentiating this virus from that described by Baker [1943] as causing diarrhea in calves) Buddingh and Dodd (1944) investigated the etiology of nonfatal sporadic stomatitis and diarrhea of infants. A filterable virus was detected in throat-washings and stools which was capable of causing an iritis and conjunctivitis in the eye of the rabbit. It does not appear to be herpes simplex virus on the basis of the nonkeratitic nature and the short duration of the ocular lesions in rabbits, and the fact that the sera of convalescent patients neutralize the virus but fail to neutralize herpes virus. Buddingh and Dodd also present evidence that adults in close contact with the disease may become asymptomatic temporary carriers. The relation of this and the other stool isolates described above to the better characterized enteroviruses remains obscure.

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History

The first description of the inclusions typical of this disease in man was made by Jesionek and Kiolemenoglou (1904), and successful transmission using the guinea pig virus was reported in 1926 by Cole and Kuttner. The occurrence of salivary gland inclusions was subsequently observed in other species of rodents (Thompson, 1932) and monkeys (Cowdry and Scott, 1935) Smith successfully propagated salivary gland virus (SVG) from mice (1954) and from man (1956). Independently, Rowe et al. (1956) and Weller et al. (1957) recovered strains of SGV from human adenoids and liver biopsies, respectively, and the latter authors demonstrated its presence in the urine of infants with hepatosplenomegaly. Finally, Rowe (1958) has isolated SGV from the salivary gland of an African monkey. This virus is antigenically related to human strains.

Clinical Picture

The anatomic and clinical manifestations of SGV infection are varied. In young infants the disease appears as a generalized blood dyscrasia associated with hepatomegaly and hepatic damage. Cerebral calcification and chorioretinitis have been reported (Weller et al., 1957). The disease is generally fatal at [illegible]

and renal damage, pneumonia and cerebral lesions are commonly observed It is frequently secondary to another illness and has been found with pertussis and fibrocystic disease of the pancreas (Smith and Velios, 1950). Although diagnosed cases in adults have been rare, it appears probable that sub-

during the infection which usually precedes the polyneuritic attack Outbreaks of the disease do occur, chiefly in the colder months Persons of all ages may be attacked, but the morbidity is highest in those between 20 and 30 years of age; both sexes are equally affected Diagnosis is made on clinical findings, particularly a protein-cell dissociation in the cerebrospinal fluid There is no specific treatment or control measure

HEMORRHAGIC MENINGO-ENCEPHALITIS

(SYNONYMS: Strumpell's disease, acute epidemic leukoencephalitis)

Acute primary hemorrhagic meningo-encephalitis is a malady characterized by predominance of large or small hemorrhagic foci and perivascular demyelination throughout the CNS It is of interest that a hemorrhagic encephalitis occurs spontaneously in horses, acute epizootic leukoencephalitis, first described in 1901 Since diagnosis is based on clinical or pathologic findings and not on specific etiology, it is impossible to say whether or not the equine disease has any connection with that of man and whether or not all the reports of the human malady concern the same disease The human malady was first noted in 1881 In western Europe in 1891, cases occurred which were regarded as secondary to influenza Thereafter, sporadic cases were observed in Russia, Australia, England, United States and elsewhere (Hurst, 1941, Margulis et al, 1944) Margulis et al (1944) reported a study of 9 Russian cases conducted during 1939-1940 CNS tissue, blood or spinal fluid from 4 patients injected intracerebrally in mice yielded a virus which the investigators regarded as the causal agent of the malady

The onset is acute with fever, anorexia, dullness, or irritability for a day or two Then follows an acute and stormy syndrome of convulsions, delirium, nuchal rigidity, coma, and death within a week, or recovery after 7 to 20 days of illness Survivors who pass into a chronic stage show frequent exacerbations, varying paralyses, ataxia and nystagmus Pupillary or ocular palsies are rare Survivors may also exhibit permanent sequelae, such as athetoid movements, paralyses and psychoses The blood count reveals a moderate polymorphonuclear leukocytosis of 10,000 to 20,000 cells per cu mm, and the spinal fluid is clear, blood-tinged, under increased pressure, with moderate pleocytosis and increased protein The pathologic picture in the CNS is one of congestion of arterioles and capillaries, the walls of many vessels are broken and surrounding tissues are infiltrated with blood The vascular walls may be necrotic, and the vessels may be filled with thrombi There are concomitant gliosis, degeneration and necrosis of neurons, and inflammatory reaction in the gray and white matter, especially in the latter, with perivascular infiltration with round and plasma cells The distinctive feature is a perivascular demyelination The diagnosis should be made guardedly, since it is based at present only on clinical and pathologic pictures that are not markedly different from those occurring generally in the postinfection encephalitides (see below) The report on the causal agent (Margulis et al, 1944) needs confirmation

Persons of all ages can be attacked, but morbidity is highest in young people Mortality rates vary in different outbreaks from 15 to 70 per cent There is no specific treatment Control measures are unknown

DURAND'S DISEASE

(SYNONYM D virus infection)

In 1940, in Tunis, during a febrile illness, Durand isolated from his blood a virus, the properties of which were studied further by Findlay (1942), who was also attacked as a result of a laboratory infection In man, after experimental inoculation, there develops after from 2 to 4 days a local cutaneous lesion associated with viremia, fever, the presence of the virus in the cerebrospinal fluid, cough, vomiting and lethargy The symptoms persist for from 6 to 8 days, after which neutralizing and complement-fixing antibodies are found in the serum. The experimental animal of choice is the guinea pig, which is susceptible by intracerebral and peripheral inoculations of virus It exhibits fever, adenopathy, enlarged spleen and pneumonitis Monkeys,

times associated cytoplasmic inclusion bodies; mental deterioration and variable neurologic signs, reflecting widespread involvement of the CNS, has been first described (Malamud et al, 1950). This form can be distinguished from herpes simplex encephalitis and from the primary demyelinating diseases Such cases are found in children and adolescents who exhibit behavior disorders, dysphasia, tremor, apraxia, rigidity, choreiform movements and finally mental deterioration The malady endures for 2 to 6 months and is often fatal The blood shows a polymorphonuclear leukocytosis of 10,000 to 20,000 cells, and the cerebrospinal fluid, the paretic type of the Lange curve, but cells and protein are normal or slightly increased, also noted in an electroencephalographic reaction of periodic nature. A viral etiology is suspected, but no definite agent has as yet been isolated

A type of infantile pneumonia with pulmonary tissue inclusions was described by Goodpasture et al. (1939) Recent study by Chany et al (1958) suggests that this may be due to infection with an adenovirus Adams (1941) reported an outbreak of pneumonia in infants in which small intranuclear inclusions were observed in autopsy specimens The author suggests that distemper or measles virus, two agents which appear to be related, was the cause of this epidemic Additional investigation of the etiology of "inclusion diseases" would be profitable

INFECTIOUS POLYNEURITIS

(Synonyms Guillain-Barré disease, Landry's paralysis, encephalomyeloradiculitis, infectious neuronitis, acute polyneuritis with facial diplegia)

Infectious polyneuritis is characterized by neurologic signs referable to the peripheral spinal and cranial nerves and sometimes by involvement of the adrenals, the liver, the heart and the kidneys The outstanding features of the condition are a protein-cell dissociation observed in the cerebrospinal fluid, progression of the neurologic symptoms, tachycardia and gastro-intestinal disturbances It occurs after diseases associated with known bacterial toxins as in diphtheria and scarlet fever, after infections of unknown etiology involving the respiratory or the intestinal tracts, and occasionally without any recognized antecedent infection The malady usually endures for several weeks or rarely may last for 3 years or longer. Except in elderly people in whom the disease may be fatal, complete recovery is the rule. However, certain patients may show permanent weakness of one side of the face or other paretic and paralytic sequelae The mortality rate is about 20 per cent, and death often occurs within the first 2 weeks usually, as a result of paralysis of the intercostal muscles. Fatal cases in children may be wrongly diagnosed as poliomyelitis There is a mild polymorphonuclear leukocytosis of 10,000 to 15,000 cells, however, in the early stages of the disease, from 25 to 80 per cent of the white blood cells may be lymphocytes (Haymaker and Kernohan, 1949) Changes in the cerebrospinal fluid are characteristic; pressure is increased, cells may be normal in numbers or at times slightly increased, the amount of protein is usually greater than normal and may at times be as much as 800 mg per 100 cc. This increase of protein may persist for a long time

There are marked edema, congestion and focal inflammation of involved nerves and spinal roots Swelling and beading of the myelin sheaths occur as well as fragmentation, beading and dissolution of axons associated with marked proliferation of the neurilemmal cells No inflammation is found in the CNS, neuronophagia is absent. Neurons in the medulla, the cord and the ganglia may exhibit changes similar to those following section or damage of axons, viz, chromatolysis, eccentricity of nucleus, vacuolization of cytoplasm, and appearance of acidophilic bodies in the cytoplasm, a picture somewhat similar to that observed by Olitsky (1939) and others in avian encephalomyelitis. Focal degeneration and necrosis associated with infiltration by mononuclear cells may be present in the adrenals, the liver, the heart and the kidneys

Attempts to transmit the disease to lower animals have failed (Sabin and Aring, 1941) The etiology is as yet unknown, although it has been considered an infective agent, perhaps a virus The studies of Sabin and Aring (1941) led them to suspect that the disease was produced by a toxic substance developed

disturbances of the type seen in parkinsonism There also may be vegetative disturbances (sialorrhea, dacryorrhea and seborrhea) or psychotic signs.

During all stages of the disease, the blood count is of little diagnostic value The cerebrospinal fluid is usually clear and shows pleocytosis (lymphocytosis), normal or slightly increased amounts of sugar, and slight increase in protein It should be emphasized that the fluid is often completely normal

Pathologic Picture

Macroscopically, the CNS may show hyperemic areas in the meninges and hyperemia accompanied by small hemorrhages in the basal ganglia, the midbrain and the pons Microscopically, the lesions of von Economo's disease are as a rule slower in developing and are of a more chronic, productive type than are those of the established viral encephalitides They are usually found in the gray matter, particularly in the mesencephalon and the diencephalon (von Economo, 1917), and are (1) degenerative and (2) inflammatory and infiltrative The former are revealed by degeneration and necrosis of neurons associated with neuronophagia; the latter are evidenced by perivascular cuffings, focal glial cell proliferation and lymphocytic infiltrations, especially in the gray matter The perivascular reaction consists of accumulation of small and large mononuclear cells and plasma cells in the sheaths of the vessels and in the perivascular spaces Hemorrhages may be seen in limited areas of the cerebral cortex, the basal ganglia, the mid-brain and the pons Demyelination is not prominent, nor are lesions in the spinal cord marked

Etiology

The cause of the malady has not been discovered, even though its general aspect conforms with that of a viral infection

Diagnosis

No laboratory tests are available at present for specific diagnosis, the disease is recognized on clinical or pathologic grounds alone, and for that reason diagnoses are not always accurate

Treatment

There is no specific treatment available.

Epidemiology

The disease has been widespread throughout the world. It has a definite seasonal incidence, occurring chiefly in winter and early spring months Thus, its spread is probably not dependent on insects It has been suggested that the malady is spread by contact or droplet infection People of all ages may be attacked, but most patients are under 40 years of age, 25 per cent of cases occur in people from 10 to 20 years old The incidence is slightly higher in males than in females. The average mortality rate is 30 per cent. Residual symptoms occur in about 20 per cent of patients Methods for prevention of spread of this disease are unknown

POSTINFECTION (DEMYELINATING) ENCEPHALITIS

(Synonyms Acute demyelinating encephalomyelitis; acute disseminated encephalomyelitis; postvaccinal [or postmeasles, etc] encephalitis; acute perivascular myelinoclasis; acute primary myelinoclasis)

Introduction

Postinfection encephalitis is an acute affection of the CNS occasionally arising during convalescence from infectious diseases, especially those caused by viruses, or following vaccination against such diseases as smallpox and rabies, or developing spontaneously in certain instances without a prior history of disease or vaccination

History

A record of demyelination [illegible]

served following smallpox, it was noted in 1886 following measles, in 1887 following preventive inoculation against rabies, and in 1907 after vaccination against smallpox Outbreaks of postvaccinal encephalitis, which assumed epidemic proportions, occurred in England and Holland during 1922 and 1924, respectively In more recent times, cases of postinfection encephalitis have been observed following several other virus diseases, especially mumps, varicella and influenza

dogs, cats, merions, mice, hamsters and voles are also susceptible. The virus is found in their blood, organs and spinal fluid; on recovery, infected hosts develop neutralizing and complement-fixing antibodies. The virus multiplies in Maitland-type tissue cultures, and chick embryos succumb after introduction of virus into the yolk sac or chorio-allantois. The virus is about 65 mμ in diameter as determined by ultrafiltration through gradocol membranes. It is preserved by glycerolation, in the frozen state, or by lyophilization

ENCEPHALITIS LETHARGICA

(SYNONYMS: Von Economo's disease; type A encephalitis, epidemic encephalitis; sleepy or sleeping sickness)

INTRODUCTION

Encephalitis lethargica is a meningo-encephalomyelitis which is probably infectious and characterized by a wide variety of signs and symptoms in different individuals and in the same person at different stages of the malady; often it is associated with ophthalmoplegia and residual parkinsonism and occurs chiefly in spring and winter months None of the names given to this malady is satisfactory Certainly many of the patients are far from being sleepy, and now it is obvious that several encephalitic viruses cause epidemics

HISTORY

It may well be that the "sleeping sickness" associated with the influenza epidemic of 1712 and "nona" of about 60 years ago may have been von Economo's disease, but the first modern cases were probably observed in 1915 in Rumania Many patients were noted in France in 1916, but the first elaborate work on the disease as it occurred in Vienna in 1917 was by von Economo (1917). It appeared in 1918 in the United States in New York City, Iowa and West Virginia (Neal et al , 1942). Epidemics were reported from different parts of the world until 1926 when they apparently ceased and have not been [illegible] specific virus has as yet been recovered

CLINICAL PICTURE

The precise incubation period is unknown, but it is assumed to be from 4 to 15 days The clinical picture as described follows that given by Barker and revised by Rivers (1943). There is a marked diversity of signs and symptoms during the course of the affection so that any type of neurologic syndrome can be simulated. In general the symptom-complex is divided into 3 stages; the first exhibits many types, but two are well defined and common· (1) a somnolent-opthalmoplegic syndrome, and (2) an irritative, hyperkinetic complex, either choreiform or myoclonic The somnolent-ophthalmoplegic type is characterized by a brief initial stage with fever, meningeal irriation, drowsiness and ocular paralyses. Other symptoms may develop, such as rigidity, paralyses of other members and psychotic disturbances. The hyperkinetic type is initiated by fever and excitement which is followed by choreiform movements or myoclonic contractions Other types seen at times during the first stage are the psychotic, with patients exhibiting a variety of signs ranging from those found in simple mental impairment to those seen in conditions simulating general paresis or schizophrenia; the poliomyelitic type with lower motor neuron paralyses, the type with involvement of posterior root ganglia; tabetic type with ataxia, an epileptomaniacal type; cataleptic type, amyostatic-akinetic form (apathy, rigidity, akinesia, amimia, slow motion and tremor); and finally, the fulminating type from which the patient succumbs within a few hours after the onset.

Of importance is the fact that during an epidemic a large number of patients may show aberrant forms and inapparent disease. In the latter case, the first suspicion of an encounter with the malady is the appearance of definite parkinsonism.

The second stage (pseudopsychoneurotic) may persist for months or years and is characterized by subjective symptoms such as headache, insomnia, irritability, dizziness and fatigue. Often objective signs of CNS lesions may be lacking.

The third stage (chronic) at times immediately follows the first and consists of motor

Louis encephalitis, the lesions are mostly in the gray matter, and neuronal damage and death are a prominent part of the picture Hurst (1944) regards the changes as nonspecific, i.e., as reactions to different agents. According to Roizin et al., (1946) and others, the lesions are in many respects similar to *those observed in other demyelinating maladies*, for example, multiple sclerosis

Etiology

Postinfection encephalitis has not been transmitted to experimental animals. There has been much speculation concerning the etiology of this demyelinating process, and the matter has been discussed at length by Hurst (1944, 1953) and Ferraro (1944)

Among the theories advanced are: (1) the infection theory, which implies that the disease is caused through action on the CNS by the virus causing the primary disease e g measles, or by the activation of a latent virus already present in the host At the present time it is safe to say that no one has definitely shown the direct action of a virus to be the cause of postinfection encephalitis Therefore, caution must be used in the interpretation of the role of the occasional virus reported to have been recovered from patients with this disease

(2) Another theory relates to toxins or poisons as the etiologic agents which are assumed to be developed during the course of the primary viral attack In this group of agents belong poisons that induce cerebral anoxia (Ferraro, 1944, Hurst, 1944) This theory was advanced because of the fact that lesions produced in the CNS by CO, KCN and tetanus toxin resemble those of postinfection encephalitis

(3) The enzyme theory assumes that myelin-destroying agents are produced in the patient or that those already present are activated by the viral infection; this theory has at present little support

(4) The theory relating to vascular thrombi has been actively advanced, especially by Putnam (1941) and his colleagues. Encephalitis and plaques of demyelination have been produced experimentally by the intravenous injection of oil or various blood coagulants into animals.

(5) The idea that phenomena of immunity or allergy play a role in the causation of postinfection encephalitis arose as the result of experimental investigations. Demyelination and inflammation of the CNS of monkeys was produced by the intramuscular injections of emulsions of normal rabbit brain tissue (Rivers et al., 1933). This work has been expanded by Morgan (1949) and by Kabat et al (1947) who injected intramuscularly into monkeys a mixture of normal monkey-brain tissue, dead tubercle bacilli and oil, and by others who successfully used rabbits, guinea pigs and mice (Olitsky and Yager, 1949) The view that the pathologic processes in postinfection encephalitis are the expression of phenomena of allergy or immunity receives support from the fact that encephalitis sometimes follows the use of immune sera or vaccines or develops in the course of antirabic treatment (Rivers 1932, Findlay, 1940; Putnam 1941, Ferraro, 1944, Hurst, 1953).

Diagnosis

Diagnosis is ordinarily made from clinical findings and a history of convalescence from a viral infection or of having been vaccinated recently Postinfection encephalitis should be distinguished from an encephalitis that may occasionally arise during the course of a viral disease for example, measles and mumps. In the latter case, the encephalitis may be caused by the virus responsible for the primary disease and the pathologic picture is wholly different from that seen in the demyelinating postinfection encephalitides Therefore, the diagnosis is difficult, and confirmation can be had only by finding the characteristic perivascular demyelination in the CNS at necropsy The disease should also be differentiated from

other encephalopathies listed by Roizin et al (1946), who believe that from a histopathologic point of view all types of demyelinating diseases, including postinfection encephalitis and acute hemorrhagic meningoencephalitis (see above), belong to one group of primary demyelinating processes, and that differences among them can be ascribed to age of the patient, duration of the affection, distribution of lesions, and degrees of host resistance

Clinical Picture

The clinical picture seen in encephalitis that develops during convalescence from viral infections depends on the type, the extent and *the location of the lesions in the CNS*. In general, it is encephalitic, myelitic, or encephalomyelitic. The encephalitic form is commonly seen following vaccination against smallpox, the myelitic type usually occurs after antirabic treatment, and both kinds are seen with equal frequency after variola. Most cases of postinfection encephalitis follow measles and vaccination against smallpox, and the picture seen under these conditions is described in detail.

Postvaccinal encephalitis usually develops abruptly from 2 to 24 days (average 10 days) after vaccination. In the encephalitic type, fever, headache, vomiting and drowsiness occur, which may be followed by photophobia, delirium, convulsions, trismus, paralyses, transient muscular weakness, in-co-ordination, ataxia, and a variety of disturbances of deep and superficial reflexes. In the myelitic type, paralyses and sensory disturbances are usually present. The course of the disease is fairly rapid and terminates in recovery within 7 to 14 days, or in the death of from 37 to 58 per cent of the patients. In most instances recovery is complete; this is a striking feature of postvaccinal encephalitis when compared with *postmeasles encephalitis, which* not infrequently is followed by sequelae.

The first indication of the development of postmeasles encephalitis is usually noted after the rash has disappeared, 4 to 6 days after defervescence. In infants, the first sign of CNS involvement may be convulsions. In older children, the disease is ushered in by drowsiness, stupor, meningismus, and con*vulsions at times. Muscular twitchings, rigid*ity, choreiform movements, ataxia, spastic paralyses, aphasia and psychic disturbances may be observed. Of particular note is the occurrence of cerebellar ataxia and, in certain instances, flaccid paralyses. Infants and children usually recover, but sequelae, such as spastic paralysis, tremors, choreic or athetoid movements, and psychic derangements may *occur.*

Encephalitis rarely follows German measles but, when it does, the clinical picture is not essentially different from that of postvaccinal encephalitis. Such is also true of the rare cases of encephalitis following dengue, smallpox and yellow fever. The encephalitis *that follows varicella is more common and is* somewhat similar to postmeasles encephalitis, i.e., cerebellar forms are prominent. Postmumps encephalitis must be differentiated from mumps meningitis and mumps meningoencephalitis. The former disease is not caused by the direct action of mumps virus, and perivascular demyelination is prominent in the pathologic picture, while the latter condi*tions are due to the direct action of mumps* virus on CNS tissue, and perivascular demyelination is not a part of the pathologic changes observed. The symptomatology and the course of the demyelinating encephalitis, which follows antirabies vaccination and bacterial infections or which arise spontaneously, are similar in most respects to the postinfection encephalitis that follows viral infections.

The cerebrospinal fluid of patients with postinfection encephalitis is clear, under increased pressure, free from infective agents, contains a normal amount of sugar and a slightly increased amount of protein and usually reveals a lymphocytic pleocytosis which may be as high as 300 cells.

Pathologic Picture

[illegible]

vascular lesions and thrombus formation which may be seen most frequently in small vessels. Hemorrhages and perivascular infiltration with lymphocytes and glial cells are found; the glial cells exhibit proliferation and active phagocytosis of fat and myelin material. A gliosis is also found diffusely scattered throughout the CNS. Degeneration and necrosis of neurons are not marked. One or another of the lesions may predominate or may be absent in any single case (Findlay, 1940). Therefore, the main features are the peri*vascular demyelination in the gray and white* matter of the brain and the cord, which may occur with or without hemorrhage, and only a slight amount of neuronal degeneration and necrosis. Rivers (1932) holds that the pathologic changes in postinfection encephalitis are not those usually produced by the direct action of a virus on the CNS where, as in St.

following World War I the disease ceased to be recognized, but it reappeared in epidemic form on the eastern European front in World War II, particularly in Jugoslavia and the Ukraine. Body lice collected in Mexico City have been found to be infected by rickettsia resembling *R. quintana* (see below), and inoculation of volunteers with louse feces produced a disease in them resembling trench fever (Varela et al, 1954).

Trench fever (Swift, 1919-1920) was characterized by sudden onset, chills, headache, dizziness, postorbital pain, nystagmus, injection of conjunctivae, severe pains in legs and back, relapsing fever, tachycardia, large spleen, and several crops of erythematous macules or papules on the chest, the abdomen and the back About one half of the patients had only one bout of fever, while the others had from 3 to 8 relapses. The incubation period in most of the human volunteers ranged from 14 to 30 days Most patients recovered in 5 or 6 weeks, others were sick for several months and in some instances for a year or two White blood cell counts were not characteristic and varied from 4000 to 27,000 per cu mm The disease was never fatal, and what is known concerning the pathologic picture was determined from biopsies of skin which showed inflammation around the small blood vessels without involvement of the walls of the vessels as is the case in typhus fever and Rocky Mountain spotted fever.

Several commissions (Strong et al, 1918) were appointed to investigate trench fever, and the combined results showed that none of the usual laboratory animals was susceptible to the disease By use of human volunteers, it was demonstrated that the etiologic agent was present in blood, sputum and urine. In 3 of 5 experiments the agent was passed with difficulty through Chamberland L filters It resisted a temperature of 60° C moist heat for 30 minutes but was inactivated by a temperature of 70° C. moist heat for a similar length of time. The body louse, *Pediculus humanus*, var. *corporis*, was shown to be a vector; it became capable of transmitting the disease 5 to 10 days after having fed upon a patient The etiologic agent was demonstrated in the lice and in their feces Since the disease was transmitted by bites, it is obvious that the infectious agent was either in the saliva or in the material regurgitated during the process of biting. Human volunteers were also infected by bringing the causative agent in contact with abraded skin. Once a louse had been infected, it excreted the active agent for the remainder of its life, which was not shortened as a result of the infection. The agent was not transmitted to larvae through eggs. Small bodies similar to rickettsiae were found in the guts of infected lice and in their feces and were not present in the absence of infection Many observations of this kind induced most investigators to place trench fever in the rickettsial group of diseases The etiologic agent was picked up from patients by lice during the first few weeks of the disease. A patient who had completely recovered was no longer a source of danger. Some patients had a chronic infection, and in several instances lice became infected by biting such patients as late as the 300th and the 443rd day after onset of illness The immunity that developed was considered to endure for only a short time, viz, several months

Mooser and his associates (Mooser et al., 1948, 1949) who investigated trench fever as it occurred in World War II, appear to have substantiated the rickettsial nature of the disease. They were able to isolate a strain of rickettsia, designated as *R. quintana*, by feeding lice on a trench fever patient in Jugoslavia Although attempts to infect mice, rats, guinea pigs, hamsters, embryonated eggs or tissue cultures with the agent were fruitless, lesions could be produced by the inoculation of infected lice intracutaneously or into the anterior optic chamber of rabbits When louse feces containing *R. quintana* were rubbed into the scarified skin of human volunteers a disease identical with trench fever resulted Mooser and his associates prepared an attenuated skin-test antigen from saline suspensions of desiccated infected intestinal tracts of lice Intradermal inoculation of a nonimmune person with this material resulted in a vesicle at the site which developed into an escharlike scab and persisted for many weeks. The lesion was frequently accompanied by a rickettsemia and clinical trench fever. Persons recovered from trench fever developed only a transient papule Subsequently, Mooser and Weyer (1953) were able to produce a subclinical infection in rhesus monkeys inoculated intravenously with a suspension of louse in-

to the causal agent. Therefore, the differences may be apparent, not real (Ferraro, 1944; Hurst, 1944, 1953).

Treatment

There is no specific treatment. On the hypothesis that encephalomyelitis may arise on a basis of hypersensitivity, ACTH or cortisone has been tested in therapy (Selling and Meilman, 1955) Prompt improvement has been observed in some cases.

Epidemiology

The occurrence of postinfection encephalitis has no relationship to the severity of the primary viral attack or to the immediate reactions after vaccination against smallpox or rabies In postvaccinal encephalitis, moreover, there is no relationship to the strain of vaccine virus used Encephalitis rarely follows the second or subsequent vaccinations against smallpox; there is no relation between morbidity and the amount of vaccine virus used or

fants or adults, e g , the largest number of cases occur in children from 2 to 14 years of age In England 93 cases of postvaccinal encephalitis were observed between 1922 and 1928, in Holland 150 between 1924 and 1928; in the United States, 71 between 1923 and 1932. Up to 1943, more than 200 cases of postmeasles encephalitis were recorded

the material used, 1 of 17,020 persons receiving antirabic vaccination with heat-inactivated virus exhibited paralysis (Casals, 1945), while in China, 5 of 201 persons who were vaccinated developed neurologic syndromes, and two of them died The general

Attention has been directed to the harmful effects on the CNS of the unborn young of maternal virus infections which occur in the early months *of pregnancy; especially,* in the case of rubella during the first 2 or 3 months It is assumed that the fetal tissues, because they are embryonic, are more susceptible. Among other viral infections implicated are rubeola, varicella, vaccinia and mumps, poliomyelitis and equine encephalomyelitis. The neurologic and mental signs shown in infants are optic atrophy, strabismus, deaf-mutism,

normal children. An interesting phase of this problem has been brought into light by Japanese and American observers (Burns, 1950). A congenital infection in swine with Japanese B encephalitis virus is described which results in stillbirth or death during the neonatal period; marked lesions of encephalitis are noted in the young, but the sows present no or negligible evidence of infection.

With respect to vaccination of pregnant women against smallpox, it would appear that risk of fetal complications exists especially if vaccination is performed in the first trimester when 24 per cent lost their fetuses as against 3 and 2 per cent loss, respectively, in the 2nd and the 3rd periods (MacArthur, 1952)

Control Measures

Since the cause of postinfection encephalitis *has not been definitely established, it is difficult* to say much about control measures. Vaccines made from nervous tissues, and particularly those containing nervous tissue to which killed tubercle bacilli and oil have been added, should be used with great care (Rivers et al., 1933; Morgan, 1947; Kabat et al, 1947) Since infants are least often attacked by postvaccinal encephalitis, primary vaccina*tion against smallpox should be performed at* the age of about 6 months and revaccination on entering school.

TRENCH FEVER

(Synonyms: Wolhynian fever, His-Werner disease, shin fever, shank fever, 5-day fever)

Trench fever was unknown until 1915, but during World War I it involved at least 1,000,000 men With *the exception of influenza,* it caused the loss of more man-days in the armed forces than did any other sickness. It is believed to have come from Russia and is known to have occurred in England, France, Flanders, Salonica, Mesopotamia, Italy, Germany and Austria In the years immediately

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testines containing *R. quintana*. By intrarectal inoculation of lice it was possible to establish that a prolonged rickettsemia developed, and Mooser and Weyer conclude that the rhesus monkey is a satisfactory animal for the experimental study of this highly fastidious agent.

Although as yet untried in trench fever, the present armamentarium of insecticides, e g, DDT, for louse control, together with the effectiveness of several antibiotics in rickettsial diseases, provide promising methods for control and treatment; thus, it appears unlikely that the disease will appear again in serious epidemics

REFERENCES

(For complete references, see also 2nd edition, Chapter 39)

Foot-and-Mouth Disease

Cartwright, B F, Pay, T W, and Henderson, W M, 1957, Multiplication of the virus of foot-and-mouth disease in culture, J Gen Microbiol *16*, 730-748

Flaum, A, 1939, Foot-and-mouth disease in man, Acta path et microbiol scandinav *16*, 197-213

Frenkel, H S, 1950, Research on foot-and-mouth disease II The cultivation of the virus in explanations of tongue epithelium of bovine animals Am J Vet Res *11*, 371-373

Olitsky, P K, Traum, J, and Schoening, H W, 1928, Report of the Foot-and-Mouth Disease Commission of the United States Department of Agriculture, U S Dept Agric Technical Bull No *76*, 1-172 (p 93)

Sellers, R F, 1955, Growth and titration of the viruses of foot-and-mouth disease and vesicular stomatitis in kidney monolayer tissue cultures, Nature, London *176*, 547

Waldmann, O, *1938, Riemser Maul-und Klauenseuche* (M K I) Vakzine, Deutsche Tierarztl Wchnschr *46*, 569

Newcastle Disease

Anderson, S G, 1946 A note on two laboratory infections with the virus of Newcastle disease of fowls, M J Australia *1*, 371

Bang, F B, 1946, Filamentous forms of Newcastle virus, Proc Soc Exper Biol & Med *63*, 5-7

Brandly, C A, Moses, H E, Jungherr, E L, and Jones, E E, 1946, Isolation and identification of Newcastle disease virus, Am J Vet Res *7*, 289-306.

Burnet, F M, 1943, Human infection with the virus of Newcastle disease of fowls, M J Australia *3*, 313-314

———, 1945, Haemagglutination by mumps virus relationship to Newcastle disease and influenza viruses, Australian J. Sc. *8*, 81-83

Burnet, F M, and Anderson, S G, 1946, Modification of human red cells by virus action II Agglutination of modified human red cells by sera from cases of infectious mononucleosis Brit J Exper Path *27*, 236-244

Cunha, R, Weil, M. L, Beard, D, Taylor, A R, Sharp, D G, and Beard, J W, 1947, Purification and characters of the Newcastle disease virus (California strain), J Immunol *55*, 69-89

Evans, A S, 1955, *The interaction of serum with* erythrocytes modified by Newcastle disease virus, J Immunol *74*, 391-396

Florman, A L, 1949, The agglutination of human erythrocytes modified by treatment with Newcastle disease and influenza virus, J Bact *57*, 31-38

Ginsberg, H S, and Horsfall, F L, Jr, 1949, A labile component of normal serum which combines with various viruses Neutralization of infectivity and inhibition of haemagglutination by the component J Exper Med *90*, 475-495

Granoff, A, and Henle, W, 1954, Studies on the interaction of the large and small hemagglutinating components of Newcastle disease virus with red cells, J Immunol *72*, 329-339

Howitt, B F, 1950, A nonspecific heat-labile factor in the serum neutralization test for Newcastle disease virus, J Immunol *64*, 73-84

Jungherr, E, Luginbuhl, R E, and Kilham, L, 1949, Serological relationships of mumps and Newcastle disease virus, Science *110*, 333-334

Kilham, L, Morgan, C, and Wyckoff, R W G, 1951, The electron microscopy of chick embryo membranes infected with Newcastle disease, J Immunol *67*, 523-528

Tyrrell, D A, 1955, New tissue culture systems for influenza, Newcastle disease and vaccinia viruses, J Immunol *74*, 293-305

Wenner, H A, Monley, A, and Todd, R N, 1950, Studies on Newcastle disease virus encephalitis in rhesus monkeys, J Immunol *64*, 305-321

Yatom, J, 1946, Conjunctivitis caused by virus of Newcastle disease, J A M A *132*, 169

Equine Infectious Anemia

Dreguss, M, and Lombard, L S, 1954, Experimental Studies in Equine Infectious Anemia, Philadelphia, *Univ Penn Press*

Kral, F, 1949, Equine infectious anemia, Vet Ext Quart Univ of Pennsylvania *116*, 14-55

Peters, J T, 1945, Equine infectious anemia transmitted to man, Ann Int Med *23*, 271-274

Reagan, R L, Lillie, M G, Hauser, J E, Poelma, L J, and Brueckner, A L, 1948, Preliminary report on the propagation of the virus of equine infectious anemia in rabbits, Proc U. S Livestock Sanitary Assn 52nd Meeting, 37

Stein, C F, Osteen, O L, Mott, L O, and Shahan, M S, 1944, Experimental transmission of equine infectious anemia by contact and body secretions and excretions, Vet Med *39*, 46-52

Cat-Scratch Disease

Baker, J A, 1944, A virus causing pneumonia in cats and producing elementary bodies, J Exper Med 79, 159-172

Daniels, W B, and MacMurray, F G, 1952, Cat scratch disease, non-bacterial regional lymphadenitis a report of 60 cases, Ann Int Med 37, 697-713

Debré, R, and Job, J C, 1954, La lymphoreticulose bénigne d'inoculation, Acta Paediat 43, Suppl [redacted]

Greer, W E R., and Keeler, C S, 1951, Cat scratch fever A disease entity New England J Med 244, 545-548

Mollaret, P., Reilly, J, Bastin, R, and Tournier, P, 1951, La decouverte du virus de la lymphoréticulose bénigne d inoculation, Presse med 59, 681-682, 701-704

——, 1956, Le virus de la lymphoréticulose bénigne d'inoculation, Presse méd 64, 1177-1180

Stevens, H, 1952, Cat-scratch fever encephalitis, Am J Dis Child 84, 218-222

Lymphocytic Choriomeningitis

Adair, C V, Gould, [redacted] L, and Smadel, J E, 1953, Aseptic meningitis, a disease of diverse etiology clinical and etiologic studies on 854 cases, Ann Int Med 39, 675-704

Armstrong, C, 1940-1941, Studies on choriomeningitis and poliomyelitis, Harvey Lectures 36, 39-65

Armstrong, C., and Lillie, R D, 1934, Experimental lymphocytic choriomeningitis of monkeys and mice produced by a virus encountered in studies of the 1933 St Louis encephalitis epidemic, Pub Health Rep 49, 1019-1027

Haas, V H, and Stewart, S E, 1956, Sparing effect of A-methopterin and guanazolo in mice infected with virus of lymphocytic choriomeningitis, Virology 2, 511-516

Hammon, W M, 1956, Encephalitis in Diagnostic Procedures for Virus and Rickettsial Diseases, ed 2, pp 169-206, New York, Am Pub Health Assoc

Janeway, C H, and Farmer, T W, 1942, Infections with the virus of lymphocytic choriomeningitis, Medicine 21, 1-37

Lepine, P, Mollaret, P, and Kreis, B, 1937, Réceptivité de l'homme au virus murin de la choriomeningite lymphocytaire Reproduction expérimentale de la meningite lymphocytaire benigne Compt rend Acad sc 204, 1846-1848

Lillie, R D, 1950, The pathlogy of lymphocytic choriomeningitis virus infection a discussion in Kidd, J G (ed), The Pathogenesis and Pathology of Viral Diseases, pp 181-193, New York, Columbia Univ Press

Rivers, T M, and Scott, T F M, 1935, Meningitis in man caused by a filterable virus, Science 81, 439-440

Rowe, W P, 1954, Studies on pathogenensis and immunity in lymphocytic choriomeningitis infection in the mouse, U S Naval Med Res Instit Rep 12, 167-220

Shaughnessy, H J, and Milzer, A, 1939, Experimental infection of Dermacentor andersoni Stiles with the virus of lymphocytic choriomeningitis, Am J Pub Health 29, 1103-1108

Shwartzman, G, 1944, Recovery of the virus of lymphocytic choriomeningitis from the erythrocytes of infected animals, J Immunol 48, 111-127

Smadel, J E, Green, R H, Paltauf, [redacted] M, and Gonzales, T A, 1942, Lymphocytic choriomeningitis two human fatalities following an unusual febrile illness, Proc Soc Exper Biol & Med 49, 683-686

Smadel, J E, and Wall, M J, 1941, Identification of the virus of lymphocytic choriomeningitis, J Bact 41, 421-430

Syverton, J T, McCoy, O R, and Koomen, J, 1947, The transmission of the virus of lymphocytic choriomeningitis by Trichinella spiralis, J Exper Med 85, 759-769

Traub, E, 1935, A filterable virus recovered from white mice, Science 81, 298-299

Wallgren, A, 1925, Une nouvelle maladie infectieuse du système nerveux central? Acta paediat 4, 158-182

Encephalomyocarditis

Bedung, R, and Koch, F, 1952, Versuch einer Differentialdiagnose der Abakteriellen Meningitis, Zts chr Kinderh 72, 85 93

Beller, K, and Keller, W, 1949, Zur Atiologie Akut Entzundlicher Erkrankungen des Zentral-Nervensystems, in Besonderen der Poliomyelitis, Klin Wchnschr 37, 422 431

Colter, J S, Bird, H H, and Brown, [redacted] A, 1957, Infectivity of ribonucleic acid from Ehrlich ascites tumour cells infected with Mengo encephalitis, Nature, London 179, 859-860

Dick, G W A, 1949, The relationship of Mengo encephalomyelitis, Encephalomyocarditis, Columbia-SK and M M viruses, J Immunol 62, 375-386

Gajdusek, C, 1955, Encephalomyocarditis infection in childhood, Pediatrics 16, 819-824

Gard, S, and Heller, L, 1951, Hemagglutination by Col-MM-virus, Proc Soc Exper Biol [redacted] Med 76, 68-73

Hallauer, C, 1949, Agglutination von Hammelerthrocyten durch murine Poliomyelitisvirusstamme, Proc 4th Int Cong Microbiol, pp 257, 1947, Copenhagen

—— 1951 Die Haemaeglutination muriner Poliomyelitisvirusstamme, Arch ges Virusforschg 4, 224-248

Horgan, E S, 1951, Virus Research Institute, Annual Report, 1950, p 6, East Africa High Commission, Nairobi

Jungeblut, C W 1950, Neutralization of Columbia SK and Yale SK virus by polio-convalescent and normal human sera, Arch Pediat 67, 519 530

——, 1958, Columbia SK Group of Viruses Handbuch der Virusforsch III Band Vienna, Springer In press

Jungeblut, C W, and Bautista, G, 1954, Antibodies against Columbia-SK in Mexican sera, Am. J Trop Med *3*, 466-473.

Jungeblut, C. W., and Bourdillion, J, 1943, Electron micrography of murine poliomyelitis virus preparations, J.A.M.A *123*, 399-402.

Keller, W., and Vivell, O, 1954, *Poliomyelitisähnliche Krankheitsbilder und ihre Erreger beim Menschen*, Ergebn inn Med u Kinderh *5*, 1-96

Kilham, L, Mason, P, and Davies, J N, 1955, Pathogenesis of fatal encephalomyocarditis infection in albino rats, Proc Soc. Exper. Biol & Med *91*, 148-155

———, 1956, Host-virus relations in encephalomyocarditis virus infections, Am J Trop Med *5*, 655-663

Kissling, R E, Vanella, J. M, and Schaeffer, M, *1956, Recent isolations of encephalomyocarditis virus*, Proc Soc Exper Biol & Med *91*, 148-152

Olitsky, P K, and Yager, R H, 1949, Hemagglutination by Columbia-SK, Columbia MM, Mengo encephalomyelitis and encephalomyocarditis viruses experiments with other viruses, Proc Soc. Exper Biol & Med, *71*, 719-724

Rustigian, R, and Pappenheimer, A M, 1949, Myositis in mice following intramuscular injection of viruses of the mouse encephalomyelitis group and of certain neurotropic viruses, J Exper Med *89*, 69-92

Schmidt, E C H, 1948, Virus myocarditis Pathologic and experimental studies Am J Path *24*, 97-118

Smadel, J. E, and Warren, J, 1947, The virus of encephalomyocarditis and its apparent causation of disease in man, J Clin Invest *26*, 1197.

Verlinde, J D, and Nihoul, E, *1950, Excretion of poliomyelitis virus by healthy contact persons*, Nederl tijdschr geneesk *94*, 1186-1189

Verlinde, J D, and van Tongeren, H E, 1953, *Human infections with viruses of the Columbia* SK group, Arch ges Virusforschg *5*, 217-230

Vivell, O, and Mauer, R, 1952, Untersuchunger uber die Haemagglutination von Viren der Parapoliomyelitis Group, Ztschr f Immunitatsforsch *109*, 246-256

Warren, J, Russ, S B, and Jeffries, H, 1949a, Neutralizing antibody against viruses of the *encephalomyocarditis group in the sera of wild rats*, Proc Soc Exper Biol & Med *71*, 376-387

Warren, J, Smadel, J E, and Russ, S B, 1949b, *The family relationship of encephalomyocarditis*, Columbia-SK, M.M, and Mengo encephalomyelitis viruses, J Immunol *62*, 387-398.

Weil, M L, Warren, J, Breese, S. S, and Russ, S B, 1951, Separation of encephalomyocarditis from tissue components by means of protamine precipitation and enzymic digestion, J Bact *63*, 99-105.

Warts: Verrucae

Kingery, L. B, 1921, The etiology of common warts Their production in the second generation J.A.M.A. *76*, 440-442

van Rooyen, C. E, and Rhodes, A J, 1940, Warts (verrucae) *in* Virus Diseases of Man, pp 131-135 London, Milford

Strauss, M. J., Bunting, H, and Melnick, J. L, 1950, Virus-like particles and *inclusion bodies* in skin papillomas, J. Invest. Dermat. *15*, 433-443.

Wile, U J, and Kingery, L B, 1919, The etiology *of common warts Preliminary* report of an experimental study, J.A.M.A *73*, 970-973.

Molluscum Contagiosum

Banfield, W. G, Bunting, H., Strauss, M. J., and Melnick, J L, 1951, Electronmicrographs of thin sections of molluscum contagiosum, Proc. Soc Exper Biol & Med *77*, 843-847.

Mitchell, J C, 1953, Observations on the virus of molluscum contagiosum, Brit J. Exper. Path. *34*, 44-49

Rake, G., and Blank, H, 1950, The relationship of host and virus in molluscum contagiosum, J. Invest. Dermat *15*, 81-94

Strauss, M J, Shaw, E. W., Bunting, H, and Melnick, J. L, 1949, "Crystalline" virus-like particles from skin papillomas characterized by intranuclear inclusion bodies, Proc. Soc Exper. Biol & Med *72*, 46-50

van Rooyen, C. E, 1938, The micromanipulation and microdissection of the molluscum contagiosum inclusion body, J Path & Bact *46*, 425-436

———, *1939, The chemical composition of the molluscum* contagiosum inclusion body, J Path & Bact *49*, 345-349.

van Rooyen, C E., and Rhodes, A. J, 1940, Molluscum contagiosum *in* Virus Diseases of Man, pp 121-130, London, Milford

Epidemic Viral Gastroenteritis

Baker, J A, 1943, A filterable virus causing enteritis and pneumonia in calves, J. Exper Med *78*, 435-446.

Buddingh, G J, and Dodd, K., 1944, Stomatitis and diarrhea of infants caused by a hitherto unrecognized virus, J Pediat. *25*, 105-113

Cook, G. T., and Marmion, B P., *1947, Gastroenteritis* of unknown aetiology, Brit M J *2*, 446-450

Gordon, I, Ingraham, H. S, Korns, R. F, and Trussel, R E., 1949, Gastroenteritis in man due to a filtrable agent, New York State J Med *49*, 1918-20

Gordon, I, Patterson, P. R., and Whitney, E., 1956, Immunity in volunteers recovered from non-bacterial gastroenteritis, J. Clin Invest *35*, 200-205

Jordan, W. S, Gordon, I, and Dorrance, W R, 1953, A study of illness in a group of Cleveland families, J. Exper. Med *98*, 461-475

Kojima, S, et al, 1948, Studies on the causative *agent of the infectious* diarrhea, records of the experiments on human volunteers, Jap M J *1*, 467-481.

Light, J S, and Hodes, H L, 1943, Studies on epidemic diarrhea of the newborn isolation of filterable agent causing diarrhea in calves, Am. J. *Pub Health 33, 1451-1454.*

Reimann, H. A., Hodges, J H, and Price, A H,

1945, Epidemic diarrhea, nausea and vomiting of unknown cause, J A M A *127*, 1-6

Salivary Gland Virus Infection

Adams, J M, 1941, Primary virus pneumonitis with cytoplasmic inclusion bodies, J A M A *116*, 925-933

Birdsong, M, Smith, H E, Mitchell, F N, and Corey, J M, 1956, Generalized cytomegalic inclusion disease in newborn infants, J A M A *162*, 1305-1308

Cappell, D F, and McFarlane, M N, 1947, Inclusion bodies (protozoonlike cells) in the organs of infants, J Path & Bact. *59*, 385-398

Chany, C, Lépine, P, Lelong, M, Le Tan Vinh, Satge, P, Virat, F, 1958, Severe and fatal pneumonia in infants and young children associated with adenovirus infection, Am J Hyg *67*, 367-378

Cowdry, E V, and Scott, G H, 1935, Nuclear inclusions suggestive of virus action in the salivary glands of the monkey, *Cebus fatuellus*, Am J Path *11*, 647-658

Fetterman, G H, 1952, A new laboratory aid in the clinical diagnosis of inclusion disease of infancy, Am J Clin Path *22*, 424-425

Goodpasture, E W, Auerbach, S H, Swanson, H S, and Cotter, E F, 1939, Virus pneumonia of infants secondary to epidemic infections, Am J Dis Child *57*, 997-1011

Hartley, J W, Rowe, W P, and Huebner, R J, 1957, Serial propagation of the guinea pig salivary gland virus in tissue culture Proc Soc Exper Biol & Med. *96*, 281 285

Jesionek, W, and Kiolemenoglou, C, 1904, Ueber einen befund von protozoenartigen gebilden in den organen eines hereditrer-luetisechen Fotus, Munchen, Med Wschnschr *51*, 1905-1907

Kuttner, A G, and Wang, S H, 1934, The problem of the significance of the inclusion bodies found in the salivary glands of infants, and the occurrence of inclusion bodies in the submaxillary gland of hamsters, white mice, and wild rats (Peiping), J Exper Med. *60*, 773-792

McCordock, H A, and Smith, M G, 1936, The visceral lesions produced in mice by the salivary gland virus of mice, J Exper Med *63*, 303-310

Malamud, N, Haymaker, W, and Pinkerton, H, 1950, Inclusion encephalitis, Am J Path *26*, 133-153

Markham, F S, and Hudson, N P, 1936, Susceptibility of the guinea pig fetus to the submaxillary gland virus of guinea pigs, Am J Path *12*, 175-182

Rowe, W P, 1958, Personal communication

Rowe, W P, Hartley, J W, Waterman, S, Turner, H C, and Huebner, R J, 1956, Cytopathogenic agent [illegible]
covere[illegible]
Proc [illegible]

Smith, M [illegible], Propagation of salivary gland virus of the mouse in tissue culture, Proc Soc Exper Biol & Med *86*, 435-440

———, 1956, Propagation in tissue cultures of a cytopathogenic virus from human salivary gland virus disease, Proc Soc Exper Biol & Med *92*, 424-430

Smith, M G, and Vellios, F, 1950, Inclusion disease or generalized salivary gland virus infection, Arch Path *50*, 862-884

Thompson, M J, 1932, Intranuclear inclusions in the submaxillary gland of the rat, J Infect Dis *50*, 162-170

Weller, T H, Macauley, J C, Craig, J M, and Wirth, P, 1957, Isolation of intranuclear inclusion producing agents from infants after illnesses resembling cytomegalic inclusion disease, Proc Soc Exper Biol & Med *94*, 4-12

Infectious Polyneuritis

Haymaker, W, and Kernohan, J W, 1949, The Landry-Guillain-Barre syndrome, Medicine *28*, 59-141

Olitsky, P. K, 1939, Experimental studies on the virus of infectious avian encephalomyelitis, J Exper Med. *70*, 565-582

Sabin, A B, and Aring, C D, 1941, Visceral lesions in infectious polyneuritis, Am J Path *17*, 469-482

Haemorrhagic Meningoencephalitis

Hurst, E W, 1941, Acute haemorrhagic leucoencephalitis, M J Australia *2*, 1-6

Margulis, M, Soloviev, V D, and Shubladze, A K, 1944, Etiology and pathogenesis of acute sporadic disseminated encephalomyelitis and multiple sclerosis, J Neurol, Neurosurg & Psychiat *9*, 63-74

Durand's Disease

Findlay, G M, 1942, Durand's disease a virus infection transmissible to animals and man, Tr Roy Soc Trop Med & Hyg *35*, 303-318.

Encephalitis Lethargica

von Economo, C, 1917, Encephalitis Lethargica, Wien. klin Wchnschr *30*, 581-585

Neal, J B, et al, 1942, Encephalitis A Clinical Study, pp 1-563, New York, Grune & Stratton

Rivers, T M, 1943, Virus diseases in Cecil, R. L, Textbook of Medicine, ed 6, pp 63-66, Philadelphia, Saunders

Postinfection (Demyelinating) Encephalitis

Burns, K F, 1950, Congenital Japanese B encephalitis infection of swine, Proc Soc Exper Biol & Med *75*, 621-625

Casals, J, 1945, A current view of the rabies problem, Ann Int Med. *23*, 74-78

Ferraro, A, 1944, Pathology of demyelinating diseases as an allergic reaction of the brain, Arch Neurol & Psychiat *52*, 443-483

Findlay, G M, 1940, Nervous affections in man and animals, Proc Roy Soc Med *33*, 161-168

Haymaker, W, and Smadel, J, 1943, The pathology of the viral encephalitides Army Med Museum, Washington, D C, June (not paginated)

Jungeblut, C. W., and Bautista, G, 1954, Antibodies against Columbia-SK in Mexican sera, Am J. Trop Med *3*, 466-473

Jungeblut, C W., and Bourdillion, J., 1943, Electron micrography of murine poliomyelitis virus preparations, J A.M.A *123*, 399-402.

Keller, W, and Vivell, O, 1954, Poliomyelitisahnliche Krankheitsbilder und ihre Erreger bein Menschen, Ergebn inn Med u Kinderh *5*, 1-96

Kilham, L, Mason, P, and Davies, J N, 1955, Pathogenesis of fatal encephalomyocarditis infection in albino rats, Proc Soc Exper Biol & Med *91*, 148-155

———, 1956, Host-virus relations in encephalomyocarditis virus infections, Am J Trop Med *5*, 655-663

Kissling, R E, Vanella, J. M, and Schaeffer, M, 1956, Recent isolations of encephalomyocarditis virus, Proc Soc Exper Biol & Med *91*, 148-152

Olitsky, P K, and Yager, R H, 1949, Hemagglutination by Columbia-SK, Columbia MM, Mengo encephalomyelitis and encephalomyocarditis viruses experiments with other viruses, Proc Soc Exper Biol & Med, *71*, 719-724

Rustigian, R, and Pappenheimer, A M, 1949, Myositis in mice following intramuscular injection of viruses of the mouse encephalomyelitis group and of certain neurotropic viruses, J Exper Med *89*, 69-92

Schmidt, E C H, 1948, Virus myocarditis Pathologic and experimental studies Am J Path *24*, 97-118

Smadel, J E, and Warren, J, 1947, The virus of encephalomyocarditis and its apparent causation of disease in man, J Clin Invest *26*, 1197

Verlinde, J D, and Nihoul, E, 1950, Excretion of poliomyelitis virus by healthy contact persons, Nederl tijdschr geneesk *94*, 1186-1189

Verlinde, J D, and van Tongeren, H E, 1953, Human infections with viruses of the Columbia SK group, Arch ges Virusforschg *5*, 217-230

Vivell, O, and Mauer, R, 1952, Untersuchunger uber die Haemagglutination von Viren der Parapoliomyelitis Group, Ztschr Immunitatsforsch *109*, 246-256

Warren, J, Russ, S B, and Jeffries, H, 1949a, Neutralizing antibody against viruses of the encephalomyocarditis group in the sera of wild rats, Proc Soc Exper Biol & Med *71*, 376-387.

Warren, J, Smadel, J E, and Russ, S B, 1949b, The family relationship of encephalomyocarditis, Columbia-SK, MM, and Mengo encephalomyelitis viruses, J Immunol *62*, 387-398.

Weil, M L., Warren, J, Breese, S S, and Russ, S B, 1951, Separation of encephalomyocarditis from tissue components by means of protamine precipitation and enzymic digestion, J. Bact *63*, 99-105.

Warts: Verrucae

Kingery, L B, 1921, The etiology of common warts Their production in the second generation J A M.A. *76*, 440-442

van Rooyen, C. E, and Rhodes, A J, 1940, Warts (verrucae) *in* Virus Diseases of Man, pp 131-135, London, Milford

Strauss, M J, Bunting, H, and Melnick, J. L., 1950, Virus-like particles and inclusion bodies in skin papillomas, J Invest. Dermat *15*, 433-443.

Wile, U. J, and Kingery, L B, 1919, The etiology of common warts. Preliminary report of an experimental study. J.A.M.A. *73*, 970-973

Molluscum Contagiosum

Banfield, W G., Bunting, H., Strauss, M. J, and Melnick, J. L, 1951, Electronmicrographs of thin sections of molluscum contagiosum, Proc. Soc. Exper Biol & Med *77*, 843-847.

Mitchell, J C., 1953, Observations on the virus of molluscum contagiosum, Brit J Exper Path *34*, 44-49

Rake, G, and Blank, H, 1950, The relationship of host and virus in molluscum contagiosum, J. Invest Dermat *15*, 81-94

Strauss, M J, Shaw, E. W., Bunting, H, and Melnick, J L, 1949, "Crystalline" virus-like particles from skin papillomas characterized by intranuclear inclusion bodies, Proc Soc Exper Biol & Med. *72*, 46-50

van Rooyen, C. E, 1938, The micromanipulation and microdissection of the molluscum contagiosum inclusion body, J Path & Bact *46*, 425-436

———, 1939, The chemical composition of the molluscum contagiosum inclusion body, J Path & Bact *49*, 345-349.

van Rooyen, C. E., and Rhodes, A. J, 1940, Molluscum contagiosum *in* Virus Diseases of Man, pp 121-130, London, Milford

Epidemic Viral Gastroenteritis

Baker, J A, 1943, A filterable virus causing enteritis and pneumonia in calves, J Exper. Med *78*, 435-446

Buddingh, G J, and Dodd, K., 1944, Stomatitis and diarrhea of infants caused by a hitherto unrecognized virus, J Pediat. *25*, 105-113

Cook, G T., and Marmion, B P., 1947, Gastroenteritis of unknown aetiology, Brit M J *2*, 446-450

Gordon, I., Ingraham, H S, Korns, R F, and Trussel, R E, 1949, Gastroenteritis in man due to a filtrable agent, New York State J Med *49*, 1918-20

Gordon, I, Patterson, P. R., and Whitney, E., 1956, Immunity in volunteers recovered from non-bacterial gastroenteritis, J Clin Invest *35*, 200-205

Jordan, W S, Gordon, I, and Dorrance, W R, 1953, A study of illness in a group of Cleveland families, J. Exper. Med *98*, 461-475.

Kojima, S, et al, 1948, Studies on the causative agent of the infectious diarrhea, records of the experiments on human volunteers, Jap M J *1*, 467-481.

Light, J S, and Hodes, H L, 1943, Studies on epidemic diarrhea of the newborn isolation of filterable agent causing diarrhea in calves, Am J Pub Health *33*, 1451-1454.

Reimann, H A, Hodges, J H, and Price, A H,

Bibliographic Index

Hurst, E W, 1944, A review of some recent observations of demyelination, Brain *67,* 103-124

———, 1953, The demyelinating diseases of man and animals, Proc Roy Soc Med *46,* 887-889

Kabat, E W, Wolf, A, and Bezer, A. E, 1947, The rapid production of acute disseminated encephalomyelitis in *rhesus* monkeys by injection of heterologous and homologous brain tissue with adjuvants, J Exper Med *85,* 117-130

MacArthur, P, 1952, Congenital vaccina and vaccina gravidarum, Lancet *2,* 1104-1106

Morgan, I M, 1947, Allergic encephalomyelitis in monkeys in response to injection of normal monkey nervous tissue, J Exper Med *85,* 131-140

Olitsky, P K, and Yager, R H, 1949, Experimental disseminated encephalomyelitis in white mice, J Exper Med *90,* 213-224

Putnam, T J, 1941, Newer conceptions of postinfectious and related forms of encephalitis, Bull New York Acad Med *17,* 337-347

Rivers, T M, 1932, Relation of filterable viruses to diseases of the nervous system, Arch Neurol & Psychiat *28,* 757-777

Rivers, T M, Sprunt, D H, and Berry, G P, 1933, Observations on attempts to produce acute disseminated encephalomyelitis in monkeys, J Exper Med *58,* 39-53

Roizin, L, Helfand, M, and Moore, J, 1946, Disseminated, diffuse and transitional demyelination of the central nervous system A clinico-histopathologic study J Nerv & Ment Dis *104,* 1-50

Selling, B, and Meilman, E, 1955, Acute disseminated encephalomyelitis *treated with ACTH,* New England J Med *253,* 275-279

Trench Fever

Mooser, H, Leeman, A, Chao, S H, and Gubler, H V, 1948, Beobachtungen an Funftagfieber, Schweiz Ztschr Path u Bakt *11,* 513-522

Mooser, H, Marti, C, and Leeman, A, 1949, Beobachtungen an Funftagfieber, Hautlasionen nach *kutaner und intrakutanen Inoculation mit Rickettsia quintana,* Schweiz Ztschr Path u Bakt *12,* 476-483

Mooser, H, and Weyer, F, 1953, Experimental Infection of Macacus Rhesus with *Rickettsia quintana* (Trench Fever), Proc Soc Exper Biol & Med *83,* 699-701

Strong, R P, Swift, H F, Opie, E L, MacNeal, W J, Baetjer, W, Pappenheimer, A M, Peacock, A D, and Rapport, D, 1918, Trench Fever, Report of Commission, Medical Research Committee, American Red Cross London, Oxford University Press

Swift, H W, 1919-1920, Trench fever in The Harvey Lectures, Series 15, pp 58-86, Philadelphia, Lippincott

Varela, G, Fournier, R, and Mooser, H., 1954, Presencia de *Rickettsia Quintana* en Piojos *Pediculus Humanus* de la Ciudad de Mexico, Rev Inst salub y enferm trop *14,* 39-41

Subject Index